UNIVERSIDADE FEDERAL DE ALFENAS

THAISE CAPUTO SILVA

SUBPRODUTO DO ABETO-NORUEGUÊS (*Picea abies*) NA MODULAÇÃO DE PARÂMETROS OXIDATIVOS E INFLAMATÓRIOS DA COLITE MURINA INDUZIDA

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Dissertação apresentada como parte dos requisitos para obtenção do título de Mestre em Ciências Ambientais pela Universidade Federal de Alfenas. Área de concentração: Gestão e manejo de recursos naturais e biodiversidade.

Orientadora: Profa. Dra. Luciana Azevedo.
Coorientador: Dr. Fernando Vitor Vieira.

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" **Subproduto do abeto norueguês (*Picea abies*) na modulação de parâmetros oxidativos e inflamatórios da colite murina induzida.** "

A Banca examinadora abaixo-assinada aprova a Dissertação apresentada como parte dos requisitos para a obtenção do título de Mestre em Ciências Ambientais pela Universidade Federal de Alfenas. Área de concentração: Gestão e manejo de recursos naturais e biodiversidade.

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RESUMO

O aproveitamento de subprodutos agroindustriais como fonte de compostos bioativos tem ganhado destaque no desenvolvimento de ingredientes funcionais voltados à saúde intestinal. Nesse contexto, a serragem do abeto-norueguês (*Picea abies*) destaca-se como uma fonte promissora de galactoglucomananos e compostos fenólicos. Dessa forma, o presente trabalho teve como objetivo avaliar o potencial biológico do extrato do subproduto do abeto-norueguês (ESAN), com ênfase em suas propriedades antioxidantes e citotóxicas nas linhagens celulares Caco-2 (adenocarcinoma colorretal humano) e CCD-18Co (fibroblasto do cólon humano), bem como seus efeitos sobre a integridade da barreira intestinal em monocamadas de células Caco-2 e em sua atuação em um modelo experimental de colite aguda induzida por dextrano sulfato de sódio (DSS) em camundongos C57BL/6. Os resultados *in vitro* demonstraram que o ESAN apresenta atividade antioxidante dependente da linhagem celular, citotoxicidade seletiva para as células cancerosas (Caco-2) com índice de seletividade de 5,1 e potencial para preservar a integridade da monocamada em células Caco-2, sugerindo efeitos benéficos sobre a função de barreira intestinal. Na etapa *in vivo*, o ESAN não foi capaz de impedir a atividade da doença induzida pelo DSS, nem a ruptura da barreira intestinal ou a depleção de mucina. Entretanto, observou-se uma restauração parcial do desempenho de crescimento nos camundongos tratados. A administração de ESAN atenuou a expressão de TNF-α e IL-6 e modulou parcialmente a IL-10, ao mesmo tempo que remodelou a composição da microbiota, aumentando a presença de Bacteroidetes e a produção de ácido acético. Contudo, o ESAN, quando administrado isoladamente, promoveu aumento da translocação bacteriana em condições de homeostase, sem comprometer a permeabilidade epitelial, bem como induziu aumento parcial na expressão de citocinas pró-inflamatórias e de marcadores de estresse oxidativo, evidenciando o caráter ambivalente de seu perfil bioativo. Em conjunto, esses achados demonstram que o ESAN exerce efeitos dependentes do contexto nas interações hospedeiro-microbiota, na inflamação, no estresse oxidativo e na função de barreira, reforçando tanto o potencial terapêutico quanto os riscos inerentes dos subprodutos florestais como candidatos nutracêuticos para o manejo de DII (Doença Inflamatória Intestinal).

Palavras chave: Carboidratos; microbiota; translocação; composto fenólico; inflamação.

ABSTRACT

The valorization of agro-industrial by-products as sources of bioactive compounds has gained increasing attention in the development of functional ingredients targeting intestinal health. In this context, Norway spruce (*Picea abies*) sawdust stands out as a promising source of galactoglucomannans and associated phenolic compounds. Accordingly, this study aimed to evaluate the biological potential of the Norway spruce by-product extract (ESAN), with emphasis on its antioxidant and cytotoxic properties in Caco-2 (human colorectal adenocarcinoma) and CCD-18Co (human colon fibroblast) cell lines, as well as its effects on intestinal barrier integrity using Caco-2 cell monolayers and its activity in an experimental model of acute colitis induced by dextran sulfate sodium (DSS) in C57BL/6 mice. *In vitro* results demonstrated that ESAN exhibits cell line–dependent antioxidant activity, selective cytotoxicity toward cancer cells (Caco-2) with a selectivity index of 5.1, and the ability to preserve Caco-2 monolayer integrity, suggesting beneficial effects on intestinal barrier function. In the *in vivo* phase, ESAN did not prevent DSS-induced disease activity, intestinal barrier disruption, or mucin depletion. However, partial restoration of growth performance was observed in treated mice. ESAN administration attenuated TNF-α and IL-6 expression and partially modulated IL-10 levels, while remodelling gut microbiota composition by increasing *Bacteroidetes* abundance and acetic acid production. Notably, when administered alone, ESAN increased bacterial translocation under homeostatic conditions without compromising epithelial permeability and induced partial increases in pro-inflammatory cytokine expression and oxidative stress markers, highlighting the ambivalent nature of its bioactive profile. Collectively, these findings demonstrate that ESAN exerts context-dependent effects on host–microbiota interactions, inflammation, oxidative stress, and barrier function, underscoring both the therapeutic potential and inherent risks of forest by-products as nutraceutical candidates for the management of inflammatory bowel disease (IBD).

Keywords: carbohydrates; microbiota; translocation; phenolic compound; inflammation.

SUMÁRIO

1. INTRODUÇÃO

Todos os anos, um volume expressivo de resíduos agroflorestais é gerado pelo processamento industrial da madeira (Spinelli *et al.*, 2019). Entre esses resíduos, a serragem destaca-se como um subproduto resultante das diferentes etapas da marcenaria, sendo constituída principalmente por celulose, lignina e hemicelulose, além de diversos compostos extrativos, como ácidos orgânicos, açúcares solúveis, resinas, ceras, óleos e compostos fenólicos (Lima *et al.*, 2024; Mallakpour; Sirous; Hussain, 2021). Na maioria das vezes, esses resíduos são descartados ou destinados a aplicações de menor valor agregado, como na produção de energia por meio da queima ou na geração de biogás. Considerando que as paredes celulares vegetais constituem uma importante reserva de polissacarídeos e compostos biologicamente ativos, a recuperação dessas frações antes de sua destinação a esses processos, configura-se como uma estratégia promissora para a agregação de valor a esses materiais, contribuindo de forma significativa para os princípios da bioeconomia circular (Granato *et al.*, 2022).

A *Picea abies*, popularmente conhecida como abeto-norueguês é uma das espécies de árvores coníferas mais comuns e economicamente importantes da Europa (Huber *et al.*, 2023). Em razão do elevado volume de resíduos gerados durante o processamento de sua madeira, a serragem dessa espécie pode ser aproveitada como matéria-prima para a obtenção de galactoglucomananos, por meio da extração por água quente pressurizada, originando frações constituídas predominantemente por essas hemiceluloses (Granato *et al.*, 2022). Esse polissacarídeo apresenta propriedades semelhantes às da goma guar e de outras galactomananas, caracterizando-se como uma fibra alimentar solúvel, com resistência à digestão hidrolítica e fermentação no intestino grosso. (Lima *et al.*, 2024). Além disso, o processo extrativo promove a coextração de compostos fenólicos, tanto livres quanto ligados às hemiceluloses. Esses resíduos fenólicos contribuem para a estabilidade e capacidade emulsificante do galactoglucomanano, ampliando seu potencial de uso como ingrediente funcional em aplicações alimentícias, cosméticas e farmacêuticas (Bhattarai *et al.*, 2019; Lima *et al.*, 2024).

Evidências recentes demonstram que esses polifenóis dietéticos e fibras solúveis podem modular favoravelmente a microbiota intestinal, atuando como prebióticos ao estimular o crescimento de microrganismos benéficos e contribuir para a restauração da homeostase da mucosa (Caetano; Castelucci, 2022; Chiu *et al.*, 2021). Considerando que a disbiose tem sido associada como uma das principais causas de doenças inflamatórias intestinais por contribuir

com o aumento da permeabilidade epitelial e com a translocação de componentes microbianos potencialmente nocivos, como lipopolissacarídeos (LPS) e toxinas, desencadeando inflamação local e sistêmica (Liang *et al.*, 2024; Sánchez-Moya *et al.*, 2024), a modulação da microbiota intestinal por esses compostos emerge como uma estratégia promissora para a prevenção e o manejo dessas condições.

A colite ulcerativa (CU) é uma doença inflamatória intestinal caracterizada por inflamação crônica e recorrente da mucosa e submucosa do cólon e do reto, resultando em manifestações intestinais e extraintestinais que comprometem significativamente a qualidade de vida dos pacientes (Le Berre; Honap; Peyrin-Biroulet, 2023). Em 2023, a prevalência global da CU foi estimada em aproximadamente 5 milhões de indivíduos, com aumento expressivo da incidência e das taxas de hospitalização em países em desenvolvimento, como China e Índia, bem como na América Latina. Esse cenário torna a CU um importante desafio de saúde pública, não apenas pelo impacto significativo sobre a saúde física dos pacientes, mas também pelos substanciais encargos sociais e econômicos associados à doença (Tang *et al.*, 2025; Wangchuk; Yeshi; Loukas, 2024).

Embora existam diversas estratégias farmacológicas disponíveis para o tratamento da CU, a resposta clínica é frequentemente heterogênea e pode estar associada a efeitos adversos e a complicações em longo prazo. Dessa forma, a eficácia limitada, associada à baixa tolerabilidade e aos elevados custos dessas terapias, tem estimulado o interesse na investigação e no desenvolvimento de abordagens terapêuticas alternativas e complementares à doença (Nascimento *et al.*, 2021). Nesse contexto, a galactoglucomanana extraída da serragem, associada à presença de compostos fenólicos, como catequina e epicatequina, com reconhecidas propriedades antioxidantes e anti-inflamatórias (Bawono *et al.*, 2023; Lima *et al.*, 2024), reforça o potencial dessa matriz para atuar de forma integrada sobre mecanismos-chave da fisiopatologia das DII, incluindo o estresse oxidativo, a inflamação da mucosa e as alterações na microbiota intestinal, particularmente na CU

Assim, considerando a crescente demanda por alimentos funcionais voltados à promoção da saúde, aliada à abundância de recursos industriais ainda pouco explorados, como hemiceluloses e polifenóis extraídos da serragem, o presente trabalho teve como objetivo avaliar o potencial biológico do Extrato do Subproduto do Abeto-Norueguês (ESAN). Para isso, foram avaliados: (I) a capacidade antioxidante e o potencial citotóxico do extrato em linhagens celulares Caco-2 (adenocarcinoma colorretal humano) e CCD-18Co (fibroblasto do cólon humano); (II) os efeitos sobre a integridade da barreira intestinal em monocamadas de células Caco-2; e (III) os efeitos do ESAN em um modelo animal de colite aguda induzida por dextrano

sulfato de sódio (DSS), considerando parâmetros relacionados à composição da microbiota fecal, aos ácidos graxos de cadeia curta, aos processos inflamatórios e ao estresse oxidativo dos animais.

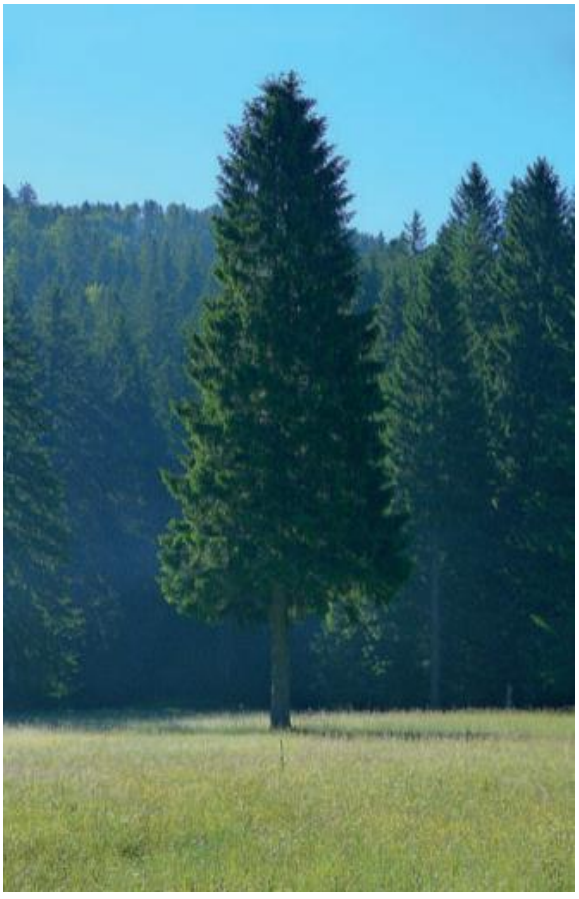
2. REFERENCIAL TEÓRICO

2.1 POTENCIAL BIOECONÔMICO DO ABETO-NORUEGUÊS

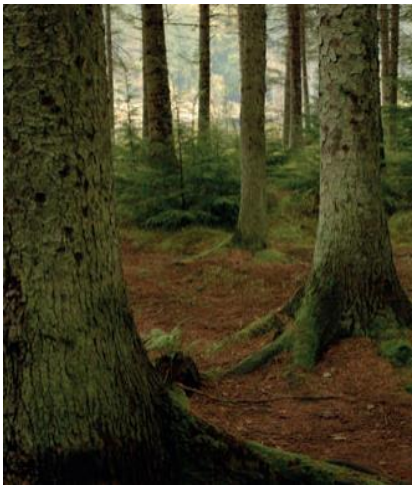
O abeto-norueguês (*Picea abies*) é uma conífera perene de grande porte pertencente à família *Pinaceae* (**Figura 1**). Trata-se de uma das espécies florestais de maior relevância ecológica e econômica da Europa, ocorrendo predominantemente na zona boreal do norte e nordeste do continente, bem como em regiões montanhosas e subalpinas da Europa central, onde é frequentemente cultivado (Caudullo *et al*, 2016; Huber *et al.*, 2023).

Figura 1. Imagens da *Picea abies*

A)



B)



C)

Fonte: Caudullo *et al*, 2016.
Legenda: A) Abeto-norueguês (*Picea abies*); B) Plantação madura do abeto-norueguês; C) Exsudato resinoso obtidos da casca do tronco e dos galhos.

Sua importância econômica está associada à versatilidade de uso de sua madeira, amplamente empregada na construção civil e na produção de papel. Além disso, o abeto-norueguês destaca-se por apresentar elevada produtividade, boa rentabilidade e regimes de

manejo florestal bem estabelecidos, respondendo por aproximadamente 23% do estoque florestal total europeu em 2020 (Huber *et al.*, 2023; Petersson *et al.*, 2021).

Do ponto de vista ecológico, sua sobrevivência em vales frios, permitida pela umidade constante do solo e pela reduzida competitividade de espécies folhosas, contribui para a adaptabilidade dos ecossistemas florestais e para a manutenção da biodiversidade, principalmente por meio da definição de nichos para organismos edáficos. Neste contexto, o acúmulo de matéria orgânica, favorecido por essa espécie, aumenta a retenção hídrica e reduz o escoamento superficial, protegendo o ambiente contra a desidratação durante períodos de seca (Samec *et al.*, 2023).

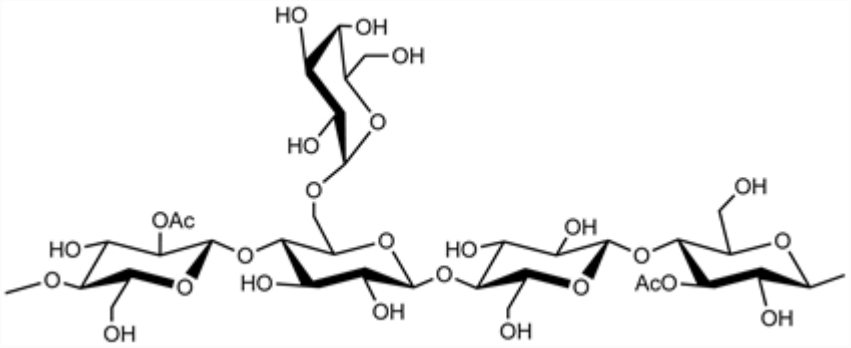
As indústrias madeireira e de celulose associadas ao processamento do abeto-norueguês geram grandes volumes de subprodutos, como casca e serragem. Esses materiais são tradicionalmente destinados à queima para geração de energia ou ao uso em aplicações de baixo valor agregado (Bhattarai *et al.*, 2019; Granato *et al.*, 2022). No entanto, a biomassa lignocelulósica derivada desses subprodutos, composta por celulose, hemiceluloses e lignina, constitui uma fonte potencial de polissacarídeos bioativos (Granato *et al.*, 2022).

Nas coníferas, a galactoglucomanana acetilada corresponde a aproximadamente 10–25% em massa dos tecidos da madeira macia, constituindo o principal tipo de hemicelulose presente nesses materiais, em especial no abeto-norueguês (Granato *et al.*, 2022; Willför *et al.*, 2008). Estruturalmente, a galactoglucomanana apresenta uma cadeia principal linear formada por unidades de β-(1→4)-D-manopiranosil e β-(1→4)-D-glucopiranosil, com ramificações laterais de α-(1→6)-D-galactopiranosil (**Figura 2**). Essa organização estrutural, associada à presença de resíduos fenólicos, é responsável pelas características anfipáticas dessas hemiceluloses, as quais viabilizam sua aplicação tecnofuncional como espessantes e estabilizantes, bem como na formação de filmes e hidrogéis, tornando os galactoglucomananos ingredientes funcionais de interesse para aplicações alimentícias, cosméticas e farmacêuticas. (Lima *et al.*, 2024; Valoppi *et al.*, 2019).

Além dos polissacarídeos estruturais, as cascas e a serragem do abeto-norueguês, constituem uma fonte relevante de compostos fenólicos. Nessas matrizes, já foram identificados flavanóis como catequina e epicatequina, além de glicosídeos flavonoides, lignanas e proantocianidinas (Lima *et al.*, 2024; Nisca *et al.*, 2021; Sut *et al.*, 2021). A aplicação de métodos de extração com solventes orgânicos, como o metanol, tem permitido o isolamento e a caracterização de diversos polifenóis, incluindo a taxifolina e seus derivados, bem como estilbenos e piceasídeos (Sut *et al.*, 2021). Além disso, a casca do tronco e dos galhos apresenta

exsudatos ricos em ácidos resínicos, terpenoides e outros compostos fenólicos, os quais ampliam a diversidade química encontrada nessas matrizes vegetais (Goels *et al.*, 2022).

Figura 2. Estrutura molecular da galactoglucomanana evidenciando a cadeia principal β-(1→4), as ramificações α-(1→6) e os grupos O-acetil.



Fonte: (Hu *et al.*, 2020)

Outras frações da biomassa de *Picea abies*, como ramos e agulhas, frequentemente subutilizadas pela indústria madeireira, também se destacam como fontes promissoras de compostos bioativos. Essa espécie acumula óleos essenciais em diferentes partes da planta, com maior abundância nas agulhas, os quais apresentam propriedades antimicrobianas (Schoss; Kočevar Glavač; Kreft, 2023; Visan *et al*., 2021).

Nesse contexto, o conceito de biorrefinaria surge como uma abordagem integrada e sustentável para a valorização dos subprodutos do abeto-norueguês, permitindo o aproveitamento sequencial e completo dessas frações. Assim, metabólitos ativos e hemiceluloses presentes na serragem ou na casca seriam extraídos e direcionados a diferentes aplicações, enquanto a fração residual da biomassa poderia ser posteriormente destinada à combustão ou gaseificação para a geração de energia, maximizando o valor agregado da biomassa e contribuindo redução de resíduos no contexto da bioeconomia circular (Granato *et al.*, 2022).

2.2 EXTRAÇÃO COM ÁGUA QUENTE PRESSURIZADA (EAQP)

A extração com água quente pressurizada (EAQP) é um método de extração sustentável que utiliza água pura mantida no estado líquido sob pressão, a temperaturas superiores ao ponto de ebulição (100 °C a 1 bar) e inferiores ao ponto crítico da água (374 °C a 221 bar), para a recuperação de compostos a partir de matrizes sólidas. Em condições subcríticas, as propriedades físico-químicas da água são alteradas, com redução da constante dielétrica para

valores comparáveis aos do metanol ou do etanol, o que possibilita a solubilização de compostos semipolares sem a necessidade de solventes orgânicos. O aumento da temperatura também reduz a viscosidade da água, o que favorece sua penetração na matriz da amostra e intensifica a transferência de massa, resultando em maior eficiência do processo de extração (Kilpeläinen *et al.*, 2014; Knierim *et al.*, 2024). Além disso, a água é um solvente ecologicamente correto, de baixo custo, amplamente disponível, passível de ser purificada e filtrada para reutilização em extrações subsequentes (Granato *et al.*, 2022; Knierim *et al.*, 2024).

Em temperaturas entre 160 e 180 °C, as hemiceluloses da madeira podem ser extraídas por EAQP. No abeto-norueguês, a temperatura de 170 °C promove principalmente a extração de galactoglucomananos, ao mesmo tempo em que reduz a solubilização de lignina. O aumento da temperatura favorece a hidrólise dessas macromoléculas e altera propriedades da água como solvente, resultando na solubilização de frações hemicelulósicas (Granato *et al.*, 2022; Kilpeläinen *et al.*, 2014).

Um estudo conduzido por Kilpeläinen et al. (2014) reportou a composição da serragem, do extrato obtido por EAQP e do resíduo sólido do abeto-norueguês, conforme apresentado na **Tabela 1**. Assim, os extratos obtidos por EAQP como o ESAN, consistem em uma mistura complexa de compostos químicos, incluindo hemiceluloses, compostos fenólicos livres e ligados às hemiceluloses, bem como lignina residual (Valoppi *et al.*, 2019). Ainda, dependendo da aplicação pretendida, a galactoglucomanana pode também ser concentrada por precipitação com etanol, método que reduz a solubilidade dos polissacarídeos e promove sua precipitação, ou, alternativamente, por etapas de centrifugação (Bhattarai *et al.*, 2019; Valoppi *et al.*, 2019).

Tabela 1. Componentes presentes na serragem, extrato e resíduo da EAQP do abeto-norueguês.

Componentes	Serragem	Extrato obtido por EAQP	Serragem extraída (resíduo)
Celulose	42%	-	58%
Hemicelulose	23%	75%	7%
Lignina	26%	17%	31%
Extrativos	3%	7%	2%
Outros compostos	6%	2%	2%

Fonte: (Kilpeläinen *et al.*, 2014)
Outros compostos: cinzas e proteínas.

2.3 DOENÇA INFLAMATÓRIA INTESTINAL: COLITE ULCERATIVA

A doença inflamatória intestinal (DII) é uma condição inflamatória crônica não infecciosa, imunomediada e recidivante que afeta o trato gastrointestinal. Seus principais tipos

são a colite ulcerativa (CU) e a doença de Crohn (DC) (Singh; Bernstein, 2022; Zhang *et al.*, 2022). A DC caracteriza-se por lesões intestinais segmentares associadas à inflamação transmural, ou seja, que acomete todas as camadas da parede intestinal (mucosa, submucosa, muscular e serosa), podendo ocorrer em qualquer segmento do trato gastrointestinal, desde a cavidade oral até a região perianal, com maior frequência no íleo terminal e nas regiões perianais. Em contraste, a CU caracteriza-se por inflamação superficial contínua, limitada a mucosa e a submucosa do cólon e do reto (Kamano *et al.*, 2023; Kobayashi *et al.*, 2020; Zhang *et al.*, 2022). Embora a localização, a distribuição e a histologia do foco inflamatório variem entre essas duas doenças, ambas compartilham características clínicas e mecanismos fisiopatológicos subjacentes semelhantes (Kang *et al.*, 2023; Zhang *et al.*, 2022).

Dados epidemiológicos indicam que a incidência anual da CU varia de aproximadamente 8,8 a 23,1 casos por 100.000 pessoas por ano na América do Norte e de 0,6 a 24,3 casos por 100.000 pessoas por ano na Europa. Embora historicamente mais prevalente em países ocidentais industrializados, observa-se um aumento expressivo da incidência e das taxas de hospitalização em países em desenvolvimento, como China e Índia, bem como na América Latina. Em 2023, a prevalência global de CU foi estimada em cerca de 5 milhões de indivíduos, configurando um desafio de saúde pública, não apenas pelo impacto significativo sobre a saúde física dos pacientes, mas também pelos substanciais encargos sociais e econômicos associados à doença (Tang *et al.*, 2025; Wangchuk; Yeshi; Loukas, 2024).

A CU apresenta prevalência semelhante entre homens e mulheres, com pico de início mais frequente entre os 20 e 40 anos de idade (Le Berre; Honap; Peyrin-Biroulet, 2023). Em geral, o início da doença é insidioso, evoluindo para sangramento retal em mais de 90% dos casos. Os pacientes podem apresentar ainda diarreia com diminuição da consistência das fezes ou constipação quando a inflamação é limitada ao reto (proctite). Outros sintomas incluem urgência retal, tenesmo, incontinência fecal, secreção de muco, evacuações noturnas, dor abdominal em cólica (geralmente localizada no quadrante inferior esquerdo), fadiga e perda de peso (Bruner; White; Proksell, 2023; Le Berre; Honap; Peyrin-Biroulet, 2023). Além disso, a CU apresenta um curso clínico caracterizado por períodos de remissão e recidiva, no qual aproximadamente 15% dos pacientes evoluem para a colectomia em decorrência da refratariedade ao tratamento clínico e/ou do surgimento de complicações, como displasia ou câncer colorretal, sangramento grave ou perfuração colônica (Danese; Fiorino; Peyrin-Biroulet, 2020).

Apesar de a CU ser caracterizada principalmente por sintomas gastrointestinais, cerca de 25% dos pacientes apresentam pelo menos uma manifestação extraintestinal, que pode

acometer diversos sistemas, incluindo o musculoesquelético, ocular, dermatológico e hepatobiliar, e apresenta forte correlação com a gravidade e a duração da doença. A artrite periférica e as espondiloartropatias estão entre as manifestações extraintestinais mais frequentemente relatadas. Além delas, manifestações oculares, como uveíte e episclerite e dermatológicas como o eritema nodoso e pioderma gangrenoso frequentemente indicam maior gravidade da doença (Tanveer; Jamil; Iqbal, 2025). Em muitos casos, essas condições podem preceder o diagnóstico de CU e impactar substancialmente a qualidade de vida dos pacientes, às vezes mais do que a própria doença intestinal (Bruner; White; Proksell, 2023; Rogler *et al.*, 2021).

2.3.1 Fisiopatologia da colite ulcerativa

A etiologia da CU é complexa e ainda não é totalmente compreendida. Os mecanismos subjacentes envolvem fatores ambientais, suscetibilidade genética, microbiota intestinal alterada e disfunção das respostas imunes. O início da patogênese da colite se dá pela ruptura da barreira intestinal e perda da homeostase da mucosa (Le Berre; Honap; Peyrin-Biroulet, 2023). Alterações na microbiota intestinal e a redução da camada de muco comprometem a integridade da barreira epitelial, facilitando o contato da microbiota com o epitélio. Esse dano é agravado por focos apoptóticos e por alterações na expressão das proteínas de junção estreita, permitindo a translocação microbiana. Como consequência, macrófagos e células apresentadoras de antígenos são ativados, levando à produção de quimiocinas que promovem o recrutamento de neutrófilos. Esses neutrófilos, que compõem a resposta imune inicial, formam armadilhas extracelulares (redes extracelulares compostas de cromatina e proteínas granulares dos neutrófilos), enquanto outras células imunes infiltram o tecido por meio de moléculas de adesão endoteliais. Monócitos infiltrantes diferenciam-se em macrófagos e passam a produzir citocinas pró-inflamatórias, como fator de necrose tumoral (TNF), IL-12, IL-23 e IL-6, favorecendo a polarização de linfócitos T auxiliares do tipo 1 (Th1) (Kobayashi *et al.*, 2020).

A dieta constitui o principal modulador da composição e da função da microbiota intestinal, de modo que desequilíbrios na ingestão de fibras, gorduras, proteínas e oligoelementos podem favorecer o desenvolvimento de disbiose intestinal. A disbiose observada em pacientes com CU caracteriza-se pela redução de bactérias benéficas, pela diminuição da diversidade microbiana e pelo aumento relativo de microrganismos patogênicos (Qian *et al.*, 2025).

Esse desequilíbrio da microbiota intestinal apresenta como principais características a redução de bactérias produtoras de ácidos graxos de cadeia curta (AGCC), a maior abundância de espécies capazes de secretar endotoxinas e induzir inflamação e a diminuição de bactérias comensais com propriedades anti-inflamatórias. (Qian *et al.*, 2025). Dessa forma, o desequilíbrio microecológico intestinal não se limita a alterações na composição da microbiota, mas envolve também mudanças nos perfis metabólicos microbianos, os quais regulam diretamente a homeostase imunológica intestinal e estão intimamente associados à gravidade da inflamação e ao prognóstico da CU (Tang *et al.*, 2025).

Em pacientes com colite ulcerativa, observa-se a depleção dos filos Firmicutes e Bacteroidetes, com destaque para a redução de espécies como *Roseburia hominis* e *Faecalibacterium prausnitzii* (pertencentes ao filo *Firmicutes)* importantes produtoras de AGCC, especialmente o butirato. O butirato constitui uma fonte essencial de energia para os colonócitos e exerce efeitos antimicrobianos e anti-inflamatórios, contribuindo para a manutenção da integridade da barreira intestinal e modulação das respostas imunes preservando assim a homeostase intestinal (Le Berre; Honap; Peyrin-Biroulet, 2023; Qian *et al.*, 2025).

Além do butirato, existem diversos tipos de AGCC, como formiato, acetato, propionato, isobutirato, valerato e hexanoato. Entretanto, o acetato, o propionato e o butirato são os mais abundantes, geralmente encontrados na proporção acetato > propionato > butirato. O acetato e o propionato também exercem efeitos anti-inflamatórios, podendo inibir a secreção de TNF-α induzida por lipopolissacarídeos (LPS) em células mononucleares de camundongos e humanos, bem como suprimir a ativação do NF-κB em células imunes por meio da inibição das histonas desacetilases (HDACs), enzimas que promovem a expressão de genes envolvidos na inflamação (Liu *et al.*, 2021).

2.3.2 Principais terapias farmacológicas na colite ulcerativa

Os principais objetivos do tratamento da CU concentra-se no controle da doença e na melhoria da qualidade de vida do paciente. A abordagem farmacológica vai depender fundamentalmente da gravidade (leve, moderada, grave), da extensão da inflamação (proctite, colite esquerda, pancolite) e da sua evolução ao longo do tempo. Nesse contexto, as estratégias terapêuticas podem ser classificadas em terapias de indução da remissão, voltadas à resolução do sangramento retal e da diarreia, associadas à recuperação da integridade da mucosa e à redução das ulcerações endoscópicas, e terapias de manutenção da remissão (Ferretti *et al.*, 2022; Yanofsky; Rubin, 2025).

As terapias à base de ácido 5-aminossalicílico (5-ASA), administradas por via oral e/ou retal, constituem o tratamento de primeira linha e são geralmente eficazes em pacientes com colite leve a moderada. No entanto, pacientes que não respondem adequadamente a essa terapia podem necessitar do uso de corticosteroides orais para indução da remissão, assim como pacientes com colite de moderada a grave (Kobayashi *et al.*, 2020; Yanofsky; Rubin, 2025).

Embora os corticosteroides sejam eficazes no tratamento da CU, seu uso prolongado está associado a piores desfechos clínicos, incluindo maior risco de colectomia. Dessa forma, corticosteroides tópicos, como o dipropionato de beclometasona e a budesonida, são preferíveis aos sistêmicos, devido ao seu perfil de segurança. Apesar de estarem associadas a efeitos adversos potencialmente graves, como mielotoxicidade, hepatotoxicidade, pancreatite e aumento do risco de câncer de pele não melanoma e linfoma, as tiopurinas (imunossupressores), como a azatioprina e a 6-mercaptopurina, constituem uma alternativa terapêutica para a manutenção da remissão em pacientes com CU dependente de corticosteroides (Kobayashi *et al.*, 2020).

As terapias avançadas para a CU são geralmente direcionadas a pacientes com doença moderada a grave ou que não apresentaram resposta adequada ao tratamento com corticosteroides e/ou tiopurinas. Essas terapias incluem anticorpos monoclonais, como agentes anti-fator de necrose tumoral (anti-TNF), inibidores de integrina e anti-interleucinas (IL), bem como pequenas moléculas sintéticas, como inibidores da Janus quinase (JAK) e moduladores do receptor de esfingosina-1-fosfato (S1PR) (Ferretti *et al.*, 2022; Yanofsky; Rubin, 2025).

Assim, embora as terapias medicamentosas tenham ampliado as possibilidades de manejo da colite ulcerativa, suas limitações relacionadas à eficácia incompleta, aos efeitos adversos e aos custos elevados (Tang *et al.*, 2025) evidenciam a necessidade de investigação e desenvolvimento de novas estratégias terapêuticas que possam auxiliar em melhores desfechos clínicos a longo prazo.

2.4 COMPOSTOS FENÓLICOS E HEMICELULOSES NA COLITE

Os compostos fenólicos são metabólitos secundários amplamente distribuídos no reino vegetal e derivados principalmente das vias do ácido chiquímico, do fenilpropanóide e da pentose fosfato. Nas plantas, esses polifenóis possuem uma ampla gama de funções fisiológicas como proteção contra patógenos, radiação UV, herbivoria, além de contribuir para o crescimento e reprodução vegetal. Do ponto de vista estrutural, os compostos fenólicos compartilham como característica comum a presença de pelo menos um anel aromático ligado

um ou mais grupos hidroxila (Razem *et al.*, 2022; Xu; Wang, 2025). Atualmente, mais de 8.000 compostos fenólicos já foram identificados, sendo classificados em ácidos fenólicos, flavonoides, lignanos, taninos e estilbenos. Essa ampla diversidade decorre das variações no número de anéis fenólicos, na posição dos grupos hidroxila e na natureza das cadeias laterais, fatores que determinam sua classificação e suas propriedades químicas e biológicas (Razem *et al.*, 2022; Sun *et al.*, 2024).

Os flavonoides constituem o grupo mais abundante entre os compostos fenólicos e caracterizam-se por um esqueleto heterocíclico do tipo C6–C3–C6, formado por dois anéis aromáticos ligados por um anel heterocíclico contendo oxigênio. Com base na posição de ligação do anel B e no grau de oxidação e de saturação do anel C, os flavonoides podem ser subdivididos em diferentes subgrupos, incluindo flavonas, flavonóis, flavanonas, chalconas, antocianinas, flavanóis (ou catequinas) e isoflavonas (Rahman *et al.*, 2021; Xu; Wang, 2025).

A catequina e a epicatequina são flavanóis isoméricos com reconhecidas atividades antioxidantes, anticancerígenas, anti-inflamatórias e antivirais. Seu potencial antioxidante decorre principalmente da presença de múltiplos grupos hidroxila, que atuam como doadores de prótons (H^+), neutralizando radicais livres, enquanto o anel aromático contribui para a estabilização dessas espécies reativas por meio de ressonância eletrônica (Bawono *et al.*, 2023; Eugène *et al.*, 2022).

Na colite, o processo inflamatório intestinal crônico está associado à geração e liberação de espécies reativas de oxigênio (ROS) e de nitrogênio (ERN) pelas células imunes infiltrantes. O estresse oxidativo, por sua vez, promove a ativação de genes associados às respostas imunes inata e adaptativa e compromete as defesas antioxidantes teciduais, contribuindo para a perpetuação e exacerbação da inflamação da mucosa intestinal. Entre os principais tipos de ROS produzidas pelas células inflamatórias estão o superóxido ($O_2^{\bullet -}$), o radical hidroxila ($^{\bullet}OH$), o radical hidroperoxila ($HO_2^{\bullet}$), o óxido nítrico (NO) e o oxigênio singlete ($^1O_2$). Essas espécies têm como principais alvos celulares os lipídios de membrana, as proteínas e o DNA, promovendo peroxidação lipídica, disfunção enzimática e danos genômicos, o que contribui para o aumento da destruição tecidual e da translocação bacteriana para a lâmina própria da mucosa, desempenhando, assim, um papel crucial no início e na progressão da doença (Muro *et al.*, 2024; Sahoo *et al.*, 2023).

O uso de compostos antioxidantes, como as catequinas, tem demonstrado resultados favoráveis na atenuação do estresse oxidativo e da inflamação observados nas doenças inflamatórias intestinais. Os efeitos resultam da atuação conjunta de múltiplos mecanismos que se influenciam mutuamente. As catequinas absorvidas pelo trato intestinal exibem diversas

atividades fisiológicas, mas as não absorvidas podem agir de modo semelhante aos prebióticos, promovendo o aumento da abundância relativa de bactérias benéficas e fortalecendo a capacidade anti-inflamatória do ecossistema intestinal, através da modulação de metabólitos microbianos como AGCC e ácidos biliares. Além disso, a ativação da via Nrf2 por esses compostos contribui para a redução da produção de citocinas pró-inflamatórias, principalmente por meio da atenuação do estresse oxidativo e da inibição de vias inflamatórias dependentes de NF-κB (Kim; Heo, 2022; Li *et al.*, 2023).

A coexistência desses flavanóis em uma estrutura polissacarídica rica em galactoglucomanana pode levar a efeitos biológicos sinérgicos no tratamento da CU. Essa hemicelulose pode ser potencialmente utilizada como fibra alimentar solúvel, semelhante à goma guar e a outras galactomananas que resistem à digestão hidrolítica e são fermentadas no cólon (Lima *et al.*, 2024). A fermentação dessas fibras leva à acidificação do conteúdo colônico e à formação de AGCC, principalmente de acetato, propionato e butirato. Os AGCC são metabolizados predominantemente por enterócitos e hepatócitos e desempenham um papel importante na homeostase intestinal aumentando a geração de células T reguladoras (Treg) e a diferenciação de células B em plasmócitos produtores de IgA. Além disso, os AGCC auxiliam na manutenção da integridade da barreira intestinal através do aumento da expressão de proteínas de junções estreitas como ZO-1 e ocludina e estimular a produção de mucina e afetar a expressão do gene produtor de muco, MUC-2 (Caetano; Castelucci, 2022; Mann; Lam; Uhlig, 2024). Assim, a natureza química complexa do Extrato do Subproduto do Abeto-Norueguês (ESAN) representa uma estratégia promissora para o manejo da colite ulcerativa, ao atuar por meio de uma abordagem multifatorial para a manutenção da homeostase intestinal e para a atenuação da progressão da doença.

3. ARTIGO: The double-edged sword effect of Norway spruce (*Picea abies*) by-product extract in colitis-induced mice

International Journal of Biological Macromolecules

The double-edged sword effect of galactoglucomannan-rich Norway spruce (Picea abies) by-product extract in colitis-induced mice

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Abstract:	Galactoglucomannan (GGM) is a hemicellulosic polysaccharide with emerging prebiotic and immunomodulatory properties. Here, we investigated the biological effects of Norway spruce by-product extract (NSBE), a forestry-derived fraction rich in GGM and phenolic compounds, in cellular systems and dextran sulphate sodium (DSS)-induced colitis in mice, assessing oxidative balance, epithelial integrity, immunomodulation, and host–microbiota interactions. In vitro, NSBE exhibited selective cytotoxicity toward colorectal cancer cells (Caco-2) and cell line–dependent redox effects, showing antioxidant activity in Caco-2 cells associated with preservation of epithelial monolayer integrity under inflammatory challenge, while inducing a pro-oxidant response in colon fibroblasts (CCD-18Co). In vivo, NSBE treatment during DSS-induced colitis did not prevent disease activity or intestinal barrier disruption, although it restored growth performance in mice. Under these conditions, NSBE administration reduced colonic malondialdehyde levels, attenuated TNF-α and IL-6 expression in the liver and colon, and modulated IL-10 production in splenocyte cultures under both unstimulated and stimulated conditions. These effects were accompanied by microbiota remodelling, characterised by increased Bacteroidetes abundance, reduced Gammaproteobacteria, and enhanced acetic acid production. Under homeostatic conditions, NSBE altered inflammatory gene expression and redox balance while increasing bacterial translocation without affecting epithelial permeability. These findings indicate that GGM-containing NSBE exerts context-dependent effects on oxidative balance, inflammation, and host-microbiota interactions, underscoring both the therapeutic potential and the inherent risks associated with the use of forestry-derived by-products as nutraceutical candidates for inflammatory bowel diseases (IBD) management.
Opposed Reviewers:	

Federal University of Alfenas – UNIFAL/MG
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March, 23th 2026

Dear Editors,

Herewith, we submit the research article entitled "**The double-edged sword effect of galactoglucomannan-rich Norway spruce (*Picea abies*) by-product extract in colitis-induced mice"** for consideration in the *International Journal of Biological Macromolecules*.

The present study describes the effects of Norway spruce by-product extract (NSBE), rich in galactoglucomannans (GGM), in a 3% DSS-induced colitis model in C57BL/6 mice and investigates the underlying cellular mechanisms related to cytotoxicity, antioxidant activity, and the extract's ability to maintain paracellular barrier function in human intestinal cell models. The findings presented here indicate that NSBE exhibits selective cytotoxicity toward colorectal cancer cells, antioxidant activity under oxidative stress conditions in Caco-2 cells, and protective effects on epithelial barrier integrity in intestinal cell models. In DSS-induced colitis, NSBE modulated inflammatory cytokine expression, reduced colonic lipid peroxidation, and reshaped gut microbiota composition, increasing *Bacteroidetes* and reducing *Gammaproteobacteria*, while enhancing short-chain fatty acid production, particularly acetate. Under homeostatic conditions, the extract induced redox and inflammatory alterations without evident tissue damage, revealing a context-dependent biological response and highlighting its potential double-edged effect.

The results presented in this study are novel and provide new insights into the use of macromolecules derived from lignocellulosic biomass residues as nutraceutical candidates for the management of inflammatory bowel diseases (IBD), particularly ulcerative colitis. Furthermore, this manuscript has not been published elsewhere and is not under consideration by any other journal.

Thank you for receiving our manuscript and for considering it for review. We appreciate the time and look forward to the response.

On behalf of all authors,

Prof. Dr. Luciana Azevedo

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To the International Journal of Biological Macromolecules

The double-edged sword effect of galactoglucomannan-rich Norway spruce (*Picea abies*) by-product extract in colitis-induced mice

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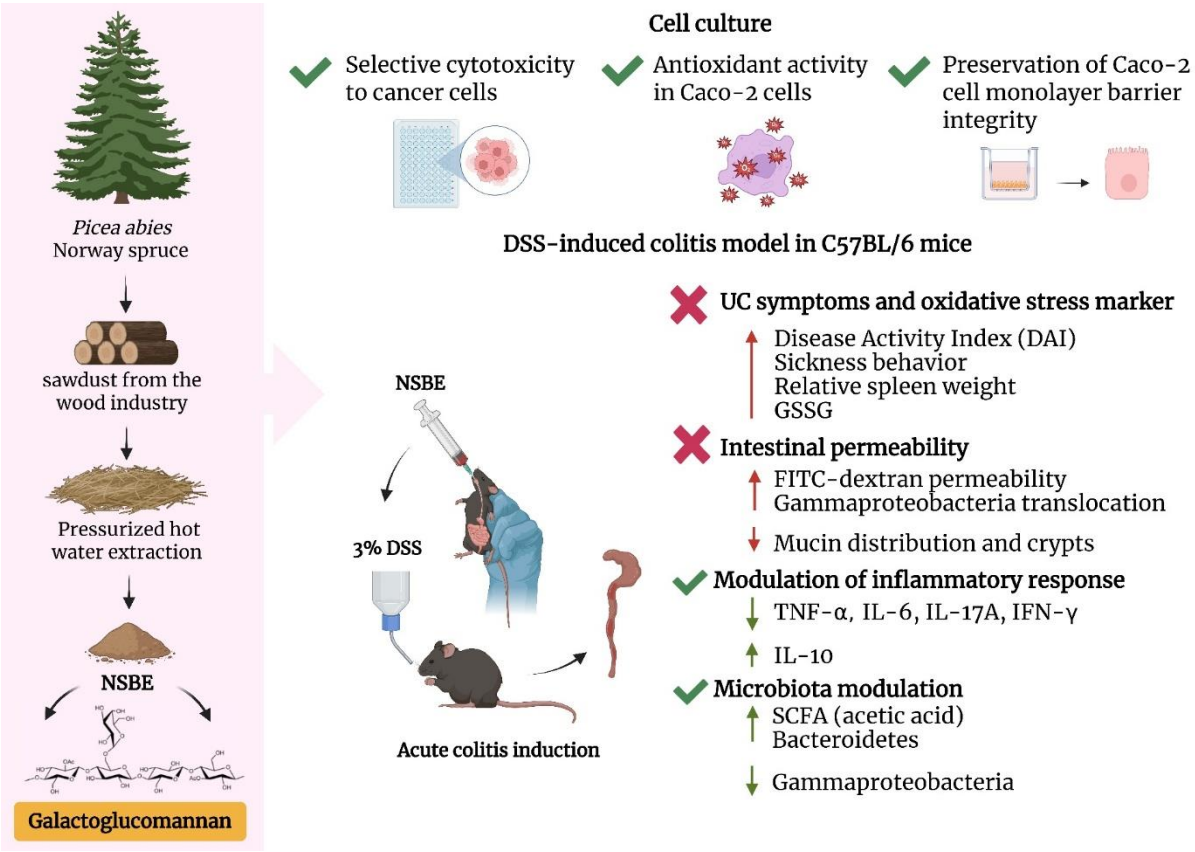
Highlights

- Norway spruce by-product extract (NSBE) showed selective cytotoxicity to cancer cells.
- NSBE exhibited antioxidant activity against H_2O_2-induced ROS in Caco-2 cells.
- NSBE preserved barrier integrity in Caco-2 monolayers challenged with LPS.
- NSBE restores total SCFAs, mainly acetate, via *Bacteroidetes* recovery.
- NSBE modulates pro- and anti-inflammatory cytokines in mice DSS-induced colitis.
- Under colitis, NSBE reshaped microbiota by reducing *Gammaproteobacteria*.

Graphical abstract



Abbreviations

ARE: Antioxidant Response Element

BMG: body mass gain

CD: Crohn's disease

DAI: disease activity index

DCFH-DA: 2′,7′-Dichlorofluorescein diacetate

DMSO: dimethyl sulfoxide

DSS: dextran sulphate sodium

FBM: final body mass

FER: feed efficiency ratio

FITC-dextran: fluorescein isothiocyanate-dextran

GAE: gallic acid equivalents

GGM: galactoglucomannans

GSH: reduced glutathione

GSSG: oxidized glutathione

IBD: inflammatory bowel diseases

IBM: initial body mass

IC_{50}: concentration reducing cell viability by 50%

IL-10: interleukin-10

IL-6: interleukin-6

IL-17A: interleukin-17A

IFN-γ: Interferon-γ

KEAP1: Kelch-like ECH-associated protein 1

LC_{50}: concentration causing 50% cell death

LPS: lipopolysaccharide

MAPK: mitogen-activated protein kinase

MGR: metabolic growth rate

MLCK: myosin light chain kinases

MPS: mononuclear phagocyte system

MTT: (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium Bromide)

MUC2: mucin 2

NRF2: Nuclear factor erythroid 2–related factor 2

NF-κB: nuclear factor-kappa B

NSBE: Norway spruce by-product extract

OFT: open field test

PAMPs: pathogen-associated molecular patterns

PBS: phosphate buffered saline

ROS: reactive oxygen species

SCFA: short-chain fatty acid

SGR: specific growth rate

SI: selective index

TDF: total dietary fibre

TEER: transcellular electrical resistance

TNF-α: tumour necrosis factor-alpha

UC: ulcerative colitis

UN: United Nations

Abstract

Galactoglucomannan (GGM) is a hemicellulosic polysaccharide with emerging prebiotic and immunomodulatory properties. Here, we investigated the biological effects of Norway spruce by-product extract (NSBE), a forestry-derived fraction rich in GGM and phenolic compounds, in cellular systems and dextran sulphate sodium (DSS)-induced colitis in mice, assessing oxidative balance, epithelial integrity, immunomodulation, and host–microbiota interactions. *In vitro*, NSBE exhibited selective cytotoxicity toward colorectal cancer cells (Caco-2) and cell line–dependent redox effects, showing antioxidant activity in Caco-2 cells associated with preservation of epithelial monolayer integrity under inflammatory challenge, while inducing a pro-oxidant response in colon fibroblasts (CCD-18Co). *In vivo*, NSBE treatment during DSS-induced colitis did not prevent disease activity or intestinal barrier disruption, although it restored growth performance in mice. Under these conditions, NSBE administration reduced colonic malondialdehyde levels, attenuated *TNF-α* and *IL-6* expression in the liver and colon, and modulated IL-10 production in splenocyte cultures under both unstimulated and stimulated conditions. These effects were accompanied by microbiota remodelling, characterised by increased *Bacteroidetes* abundance, reduced *Gammaproteobacteria*, and enhanced acetic acid production. Under homeostatic conditions, NSBE altered inflammatory gene expression and redox balance while increasing bacterial translocation without affecting epithelial permeability. These findings indicate that GGM-containing NSBE exerts context-dependent effects on oxidative balance, inflammation, and host-microbiota interactions, underscoring both the therapeutic potential and the inherent risks associated with the use of forestry-derived by-products as nutraceutical candidates for inflammatory bowel diseases (IBD) management.

Keywords: hemicellulose; microbiota; inflammation.

1. Introduction

Biopolymers such as plant cell wall hemicelluloses have attracted increasing industrial interest owing to their physicochemical, mechanical, and biological properties [1,2]. Their abundance and structural diversity differ markedly between hardwoods and softwoods [3]. Galactoglucomannans (GGM) are the predominant hemicellulose in softwoods such as *Picea abies* (Norway spruce), accounting for approximately 10–30% of their dry weight, which consists of a linear backbone of β-(1→4)-D-glucopyranosyl and partially acetylated β-(1→4)-D-mannopyranosyl units, branched with α-(1→6)-D-galactopyranosyl side groups [3,4]. In this

study, we used a Norway spruce by-product extract (NSBE) obtained from pressurised hot water extraction (PHWE) of Norway spruce sawdust at 170 °C, a process that primarily releases galactoglucomannans (GGM) together with their hydrolysis products, including oligo- and monosaccharides, as well as associated phenolic compounds [4,5].

Phenolic residues confer amphiphilic properties to GGM biomolecules, enhancing its emulsifying stability and protection against lipid oxidation compared with commonly used biopolymers such as gum Arabic and synthetic surfactants like Tween [4]. These properties enable its techno-functional applications as a thickening and stabilising agent and in bio-based films and coatings, highlighting its potential as a functional ingredient [6]. Studies on GGM extracts derived from Norway spruce have demonstrated potential immunomodulatory effects in thymocytes [7], *in vitro* prebiotic properties [5], and the ability to reduce hormone exposure-induced prostatic inflammation [8]. Focusing on NSBE, the extract exhibited antioxidant and antiproliferative properties and mitigated the development of preneoplastic lesions and morphological alterations in colonic tissue in a DMH-induced colorectal cancer model [2].

Building on these findings, chronic inflammatory bowel diseases (IBD), including ulcerative colitis and Crohn's disease (CD), constitute a key risk factor for colorectal cancer, with persistent mucosal injury, barrier disruption, and immune dysregulation driving neoplastic transformation [9,10]. Epidemiological evidence indicates a rising prevalence of IBD in newly industrialised regions of Asia, South America, and Africa, largely driven by shifts in environmental exposures and lifestyle factors [11,12]. Indeed, in 2023, ulcerative colitis affected approximately 5 million individuals worldwide [13], representing a major public health challenge not only because of its impact on patients' physical health, but also due to the considerable social and economic burdens associated with the disease [13,14].

The aetiology of UC is multifactorial, involving complex interactions among genetic, environmental, immune dysfunction, and gut microbiota dysregulation [10]. Although current therapies primarily target inflammation and modulate immune responses to alleviate symptoms, their efficacy is limited by adverse effects and long-term complications, including loss of immune tolerance and drug resistance [12,14]. Given the pivotal role of the gut microbiota in intestinal homeostasis, which comprises shaping immune responses, maintaining barrier integrity, and restricting pathogenic bacteria primarily through the production of SCFAs. Therapies that modulate microbial composition and metabolism using dietary compounds with low toxicity and multi-target actions emerge as a promising strategy to restore intestinal homeostasis and alleviate inflammation [11,15].

Considering the potential cascade use of biomass derived from Norway spruce sawdust and the increasing burden of inflammatory bowel diseases (IBD), we investigated the ability of NSBE to modulate inflammation, preserve intestinal barrier integrity, and influence microbiota composition in a murine colitis model. To elucidate the cellular mechanisms underlying these effects, we assessed its cytotoxicity, antioxidant activity, and capacity to maintain paracellular barrier function in human intestinal cell models, including colorectal adenocarcinoma cells (Caco-2) and colon fibroblasts (CCD-18Co). Together, these studies provide mechanistic insights into the potential of NSBE as a nutraceutical strategy for the management of ulcerative colitis.

2. Materials and methods

2.1 NSBE characterization and centesimal composition

The sample was obtained from Norway spruce (*Picea abies*) wood sawdust, collected in central and southern Finland, and extracted using a flow extractor (PHEW) with pressurized hot water, as described by Valoppi et al. [4]. The centesimal composition of NSBE, including moisture content, total ash, crude fat, crude protein (using a nitrogen-to-protein conversion factor of 6.25), and carbohydrates (calculated by difference), was determined according to official methods of the Nordic Committee on Food Analysis (NMKL) [16] to support the nutritional parameters of the animal study. Total dietary fibre (TDF) was quantified using the enzymatic-gravimetric method, involving sequential enzymatic digestion of a fat-free sample with heat-stable α-amylase, protease, and amyloglucosidase, followed by ethanol precipitation of the soluble fibre. Final TDF value was corrected for residual protein and ash, which were determined on the gravimetric residue [17].

2.2 Cell culture: NSBE cytotoxicity and antioxidant activity

Colorectal adenocarcinoma epithelial cells (Caco-2; BCRJ, code: 0059) and human colon fibroblasts (CCD-18co; BCRJ, code: 0400) were obtained from the Rio de Janeiro Cell Bank. Cell cultures were maintained at 37 °C in a humidified atmosphere containing 5% CO_2 and 96% relative humidity. Caco-2 cells were grown in high-glucose Dulbecco's Modified Eagle's Medium (DMEM; Sigma, CA, USA) supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic solution (10,000 U/mL penicillin and 10,000 µg/mL streptomycin; Gibco, NY, USA). CCD-18Co cells were cultured in low-glucose DMEM (Sigma, CA, USA) supplemented with 10% FBS and the same concentration of antibiotics.

To explore the cytotoxicity profile of the NSBE in cell culture, the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium Bromide) assay was used to assess the cells' viability according to Lima et al. [2]. Briefly, Caco-2 and CCD-18co cell lines were seeded in a 96-well plate (1 x 10^4 cells/well) and treated with NSBE (10 to 200 µg GAE/mL) for 48 h. Then, MTT (5 mg/mL) was applied for 4 h at 37 °C, and the formazan crystals were dissolved in dimethyl sulfoxide (DMSO). The absorbance was read at 570 nm in a microplate reader. The IC_{50} (50 % cell viability inhibition), LC50 (50 % cell death), and the Selective Index (SI) were calculated (IC_{50} normal cell/IC_{50} cancer cell).

Cellular antioxidant activity of NSBE was obtained by the DCFH-DA probe and hydrogen peroxide-induced oxidative stress [2]. First, Caco-2 and CCD-18co cells were added to the 96-well plate (6 x 10^4 cells/well,) and, after adhesion, treated with NSBE (5, 10 and 20 µg GAE/mL), hydrogen peroxide (1 mM for Caco-2 and 0.2 mM for CCD-18co; positive control), and culture medium (negative control) for 1 h at 37 °C in the dark. Then, the wells were washed with PBS 1X (Sigma-Aldrich, Sao Paulo, Brazil), and Hank's solution with H_2O_2 (150 µM) was added. The fluorescence intensity was measured at 450 nm (excitation) and 538 nm (emission). The results were expressed as a percentage of the fluorescence intensity relative to the basal cell (negative control).

Subsequently, to assess intracellular glutathione levels, cells were seeded at 0.4×10^6 per well in six-well plates and cultured until reaching 70–80% confluency. Oxidative stress was induced with H_2O_2 (1 mM for Caco-2; 0.2 mM for CCD-18Co) in the presence or absence of NSBE (5–20 µg/mL). Subsequently, cells were detached, washed twice with cold PBS, and resuspended in 300 µL of ice-cold extraction buffer (0.1% Triton X-100 and 0.6% sulfosalicylic acid in KPE). GSH and GSSG were quantified according to Rahman et al. [18].

2.3 Evaluation of FITC-dextran permeability by Caco-2 cell monolayer intestinal barrier integrity

Transepithelial electrical resistance (TEER) and fluorescein isothiocyanate–dextran (4 kDa; FITC-dextran; Sigma-Aldrich, Missouri, USA) permeability were used to assess the integrity of Caco-2 monolayers. Caco-2 cells were seeded on Transwell (12 mm diameter, 0.4 µm pore size; Corning Costar, MA, USA) at a density of 1×10^5 cells/cm² and cultured for 21 days with medium renewal every 48h until resistance values >400 Ω·cm². TEER was measured using an epithelial voltmeter before and after treatments. Cells were treated with NSBE (10 µg GAE/mL) for 24 h and subsequently exposed to LPS (10 µg/mL) for an additional 24 h in the presence of NSBE. Monolayer integrity was evaluated by adding FITC-dextran (100 µM) to

the apical compartment, followed by collection of basolateral samples after 1 and 2 h for fluorescence measurement (λ_ex 485 nm; λ_em 530 nm) [19].

2.4 In vivo experimental design and colitis-induced procedure

Six-week-old male C57BL/6 mice weighing 19 g were obtained from the animal facility of the Federal University of Alfenas and housed in polypropylene cages under controlled light (12 h light-dark cycle) and temperature 22 ± 1 °C conditions, with free access to water and commercial feed (Nuvilab CR-1, Nuvital Nutriente S/A, Colombo, Brazil). Food and water consumption, as well as the weights, were monitored daily from the start to the end of the experiment. All procedures were performed in accordance with the guidelines of the Ethics and Use of Animals Committee of the Federal University of Alfenas (#024/2024).

Mice received NSBE (400 mg/kg/day), as reported by Lima et al. [2], or vehicle (water) by oral gavage for 21 days. From day 16, all mice except the control and NSBE groups received 3% DSS (w/v; MP Biomedicals, OH, USA) in drinking water *ad libitum* for 5 days to induce colitis. The animals were randomly assigned to four groups (n = 8): Control (water gavage + water *ad libitum*), 3% DSS (water gavage + 3% DSS *ad libitum*), NSBE (NSBE gavage + water *ad libitum*), and DSS + 3% NSBE (NSBE gavage + 3% DSS *ad libitum*), as shown in **Figure 1A**. Faecal samples from each group were collected aseptically, and the animals were sacrificed immediately afterward. The colon, liver, and spleen tissues were collected for molecular and histological analyses.

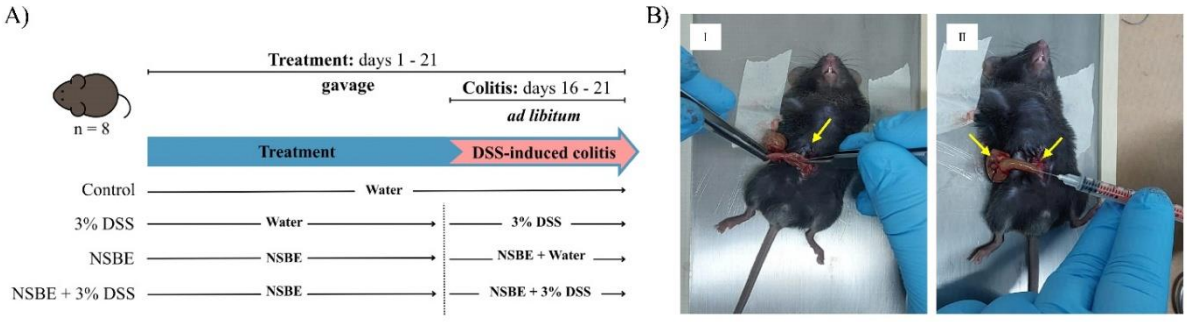


Figure 1. Experimental design and surgical procedure for the assessment of paracellular intestinal permeability in DSS-induced colitis. (A) Experimental design: Mice received NSBE (400 mg/kg/day) or vehicle (water) by oral gavage for 21 days; from day 16, colitis was induced by administering 3% DSS in drinking water *ad libitum* for 5 days. (B) Surgical procedure for the evaluation of intestinal permeability using FITC-dextran: (I) colon exteriorisation; (II) Ligation of the proximal colonic segment followed by intraluminal administration of FITC–dextran into the isolated colonic segment.

2.5 Evaluation of nutritional, clinical, and behavioural indicators of animal well-being

Body weight and growth performance were monitored daily to assess the health and metabolic status of the experimental groups. Body mass gain (BMG), feed efficiency ratio (FER), metabolic growth rate (MGR), and specific growth rate (SGR) were calculated individually and expressed as group means, as described by Prado-Silva et al. [20].

The Disease Activity Index (DAI) was calculated daily based on body weight loss, rectal bleeding, and stool consistency to assess colitis severity and the therapeutic effects of NSBE during DSS treatment. These components were scored on a 0–4 scale as follows: (I) intestinal bleeding (0 = absent; 2 = Hemoccult positive; 4 = gross bleeding), (II) body weight loss (0 = ≤1%; 1 = 1–5%; 2 = 5–10%; 3 = 10–20%; 4 = >20%), and (III) stool consistency (0 = normal; 2 = loose faeces; 4 = diarrhoea) [21]. Occult bleeding was detected using the Kastle-Mayer reagent (ACS Scientific, Sumaré, Brazil), whereas visible rectal blood was considered overt bleeding.

To further examine mucosal damage associated with DSS-induced colitis, colonic tissue from the DSS group was analysed by scanning electron microscopy (SEM). Colon fragments were longitudinally opened, rinsed with PBS, and fixed in 2.5% buffered glutaraldehyde for 24 h. Samples were dehydrated through a graded ethanol series (30–100%) [22], sputter-coated with a 15 nm gold layer, and imaged using a field-emission scanning electron microscope (FEG-SEM; MIRA4, Tescan) at 2–5 kV.

The open field test was conducted in a square arena (40 × 40 cm) at the end of the experiment (21 days), and behavioural parameters indicative of motivational state alterations, such as total distance travelled and immobility time, were quantified using EthoVision software (Noldus Information Technology, Leesburg, VA, USA) [23].

2.6 Assessment of paracellular intestinal integrity by FITC-dextran permeability in experimental colitis

Colitis-associated alterations in intestinal mucosal barrier integrity and the potential protective effects of NSBE were evaluated by measuring the translocation of FITC-dextran from the colon specifically into the bloodstream. On the final day of DSS administration, mice (n = 8) were anaesthetised with a combination of ketamine (Cetamin, ketamine hydrochloride 10%, Syntec; 100 mg/kg) and xylazine (Xilazin, xylazine hydrochloride 10%, Syntec; 10 mg/kg), and subjected to exploratory laparotomy followed by ligation of the proximal colonic segment. Then, 100 µL of FITC-dextran (2 mg/mL) was injected into the colonic lumen within the ligated segment (**Figure 1B**). After 1 h, blood was collected by cardiac puncture, centrifuged

at 3500 rpm for 10 min at 4 °C, and plasma samples were analysed for fluorescence using a microplate reader (excitation/emission 485/530 nm). FITC-dextran concentrations were calculated based on a standard curve [24].

2.7 Colon histological processing, histopathology, and microstructural analysis

The colon histological processing was performed according to Lima et al. [2], where colon fragments (collecting one section every 20 sections) were fixed in 10% buffered formalin (pH 7.2) for 24 h, dehydrated in ethanol, and embedded in glycol methacrylate histological resin. All fragments were cut at 4 μm-thick using a rotary microtome, stained with the hematoxylin and eosin method for general histopathology, and the Alcian Blue histochemical method for mucin detection (and globed cells localization). An intestinal area equivalent to 62.8 × 10^5 μm^2 was confirmed in each group in histological images obtained from twelve microscopic fields at 400*x* magnification, using brightfield microscopy (Axioscope A1, Carl Zeiss, Germany). Comparatively, colon histopathology was conducted using the vehicle-treated animals as a reference for normal microstructure, analysed by the hypertrophy of the lining epithelium, mucosa, and crypts, as well as tissue cellularity, distribution of goblet cells, and lamina propria. Mucosal thickness, lining epithelium height, crypts number per histological area, crypts depth, and crypts width were analysed as previously reported Sequetto et al. [25]. Mucin distribution was estimated using the histogram tool of the Image-Pro Plus 4.5 image analysis software and reported as relative (%) values.

2.8 Determination of glutathione redox balance and lipid peroxidation in colon and liver

Oxidative stress was assessed by measuring glutathione levels and lipid peroxidation in colon and liver tissues. Total glutathione and oxidised glutathione (GSSG) were determined according to Rahman et al. [18] using a DTNB–glutathione reductase recycling assay, with absorbance measured at 412 nm. GSH levels were calculated from total glutathione and GSSG values. Lipid peroxidation was evaluated using the thiobarbituric acid reactive substances (TBARS) assay, with absorbance measured at 532 nm and quantification based on MDA bis (dimethyl acetal) standard curve [26]. All results were normalised to the protein content of each sample, determined by the Bradford method [27].

2.9 Colon and liver cytokine gene expression by Real-Time RT-PCR

The inflammatory profile of the liver and colon was evaluated by analysing *TNF-α*, *IL-6*, and *IL-10* expression. Total RNA was extracted using TRIzol reagent (Invitrogen, Life

Technologies), and 2 µg were reverse-transcribed with Ready-To-Go RT-PCR Beads (GE Healthcare) using oligo(dT) primers. qPCR was performed on an ABI 7500 Real-Time PCR System (Applied Biosystems) with SYBR Green PCR Master Mix (Applied Biosystems) and gene-specific primers (**Table S1**). Expression levels were normalized to *β-actin* and expressed as relative units. All reactions were performed in triplicate [28].

2.10 Splenocyte culture and cytokine quantification by ELISA

The immunomodulatory effects of NSBE and DSS-induced inflammation were analysed in splenocytes obtained from treated mice. Spleens were mechanically dissociated to obtain single-cell suspensions, and erythrocytes were removed using ACK lysis buffer. The splenocytes were then resuspended in complete RPMI medium (Gibco) supplemented with 2 mM L-glutamine, 100 U/mL penicillin, 100 µg/mL streptomycin, 25 mM HEPES, and 10% heat-inactivated FBS. Cells were seeded at a density of 1×10^6 cells per well and maintained at 37 °C in a humidified atmosphere containing 5% CO_2. Cultures were stimulated for 24 h with NSBE (200 µg GAE/mL), LPS (1 µg/mL), NSBE + LPS, or RPMI alone (negative control). After incubation, cytokine levels (IFN-γ, IL-17A, and IL-10) in culture supernatants were determined by ELISA (Murine Standard ABTS Kit, PeproTech) according to the manufacturer's instructions, and absorbance was measured at 405 nm with a reference wavelength of 650 nm [29].

2.11 Faecal DNA and mesenteric lymph nodes were collected, followed by bacterial microbiota quantification by qPCR

To evaluate the impact of DSS-induced colitis and NSBE treatment on gut bacterial composition and bacterial translocation, genomic DNA was obtained from faecal samples and mesenteric lymph nodes. DNA from faecal material was extracted using the PureLink™ Microbiome DNA Purification Kit (Invitrogen, Thermo Fisher Scientific, Carlsbad, CA, USA) following the manufacturer's guidelines. Mesenteric lymph nodes were processed by mechanical disruption before DNA isolation, according to the procedure described by Wilson et al. [30]. Extracted DNA was subsequently purified using an Invitrogen spin column and quantified with a Qubit fluorometer (Thermo Fisher Scientific, Waltham, MA, USA). Bacterial populations were quantified by quantitative PCR (qPCR) using 1 µg of template DNA and primers targeting the 16S rRNA gene to detect total bacteria and specific taxa, including *Bacteroidetes*, *Firmicutes*, and *Gammaproteobacteria* (Table S1). Amplification reactions were performed in triplicate, and relative bacterial abundance was calculated using the ΔCt method,

with the water-treated group used as the reference control (ΔCt = Ct_treated – Ct_control) as previously described [2,31].

2.12 Determination of short-chain fatty acids (SCFA) in faeces

SCFA levels in faeces were quantified to assess the effects of DSS-induced inflammation and NSBE treatment on microbial metabolism. Faecal samples (30 mg) were extracted with methanol/formic acid and supernatant spiked with isoamyl alcohol, used as an internal standard. 1 µL of extract was injected into a gas chromatograph equipped with a flame ionization detector (GC-FID; Varian Chrompack CP-3800, USA) and an autosampler (Varian Chrompack CP-8400, USA) in split mode (1:10) at 250 °C. The carrier gas used was hydrogen at a constant pressure of 8 psi. The analytes (acetic, propionic, isobutyric, butyric, valeric, and isovaleric acids) were separated by a CP-Wax 52CB capillary column (60 m × 0.25 mm; 0.25 µm stationary phase thickness). Isobutyric and valeric acid were expressed as equivalents of butyric and isovaleric acid, respectively. Accuracy was determined by recovering known amounts of the standard substances added to a diluted sample (**Table S2**) The results were expressed as µmol/g in the faeces samples [32].

2.13 Statistical analysis

Non-linear regression was applied to the viability assay, and the best-fitting model was determined based on the coefficient of determination (R^2) and the p-value. Data normality was assessed using the Shapiro–Wilk test. Parametric data were analysed by one-way ANOVA followed by Tukey's post hoc test, except for splenocyte culture experiments, which were analysed by two-way ANOVA followed by Tukey's post hoc test. Histopathological data were analysed using the non-parametric Kruskal-Wallis test. Experimental data are presented as mean ± standard deviation (SD), and differences were considered statistically significant at $p \leq 0.05$. Graph generation and statistical analyses were performed using GraphPad Prism version 8.0 (GraphPad Software Inc., La Jolla, CA, USA).

3. Results and discussion

3.1 NSBE nutritional composition

The hemicellulosic fraction represents a major component of this non-conventional food ingredient and constitutes an important source of carbohydrates. Centesimal composition revealed that carbohydrates correspond to approximately 91% (91.2 g/100 g; w/w) of the total mass of NSBE, while dietary fibres comprised less than 3% of insoluble high molecular weight

and 7.5% of soluble high molecular weight. Moreover, moisture (6.7 ± 0.8 g/100 g), ash (1.7 ± 0.2 g/100 g), protein (0.4 ± 0.0 g/100 g), and fat (<0.2 ± 0.0 g/100 g) were found in smaller quantities. Taken together, this profile corresponds to 366 kcal per 100 g of lyophilized sample.

These findings complement previous analyses performed using the same NSBE batch [2,6], showing that the extract contains phenolic compounds and carbohydrate constituents, including epicatechin (177 ± 0.3 µg/g dry matter) and the monosaccharides mannose (360 ± 14 mg/g dry matter) and glucose (92 ± 4 mg/g dry matter), which are β-(1→4)-linked and form the backbone of GGM.

3.2 NSBE shows selective cytotoxic and antioxidant activity in colorectal cancer cells

Different concentrations of NSBE (10–200 µg GAE/mL) were assessed to determine its cytotoxic potential in cancer (Caco-2) and normal (CCD-18co) cells. NSBE showed a cytotoxic profile in Caco-2 cells evidenced by IC50 and LC50 (IC_{50} = 35.6 µg GAE/mL; LC_{50} = 50 µg GAE/mL), in contrast to the observed in CCD-18co cells (IC_{50} = 183.4 µg GAE/mL; LC_{50} > 200 µg GAE/mL; **Figure 2A).** These differences indicate a safety margin and selective cytotoxicity towards cancer cells, supported by a SI of 5.1 (SI > 3) [33]. Notably, at 100 µg GAE/mL, NSBE promoted a 50% increase in the proliferation of CCD-18co cells compared with untreated controls, possibly associated with increased intracellular ROS levels, suggesting adaptive redox signalling consistent with NRF2 activation [34]. Beyond its cytoprotective function, NRF2 enhances cellular metabolic capacity, which may facilitate cell cycle progression and contribute to the proliferative response observed [35,36].

Concerning the intracellular antioxidant activity data shown in **(Figure 2B-C)**, NSBE exhibited distinct behaviours between cancer and non-cancer cells. In Caco-2 cells, NSBE (5 µg GAE/mL) reduced basal ROS levels by 80% relative to the negative control. Moreover, when challenged with H_2O_2, NSBE showed a concentration-dependent protective effect against oxidative stress, with a 20 µg GAE/mL concentration restoring ROS levels to the same magnitude as those generated spontaneously. In contrast, in CCD-18co cells, NSBE exhibited a pro-oxidant profile in the absence of H_2O_2 at all concentrations tested, whereas under H_2O_2 stimulation, it showed a mild protective effect across, achieving a 40% reduction in ROS formation (20 µg GAE/mL) compared with the positive control. In previous studies [2], the undigested Norway spruce wood-sawdust extract exhibited a protective antioxidant effect in three cancer cell lines (SCC-9, HCT-8, and A549) at a concentration of 5 µg GAE/mL, while at the same concentration it enhanced the oxidative response in non-cancerous cells (HUVEC) exposed to H_2O_2. Nevertheless, extracts recovered from wood-industry byproducts appear to

exhibit cell-type-dependent activity, as they exerted antioxidant effects in human keratinocytes and normal lung cells exposed to H_2O_2 [6,37].

Glutathione (GSH) is a cytosolic tripeptide that serves as a major intracellular antioxidant. It is oxidised to GSSG in the presence of free radicals and acts as a substrate for enzymes such as glutathione peroxidase and glutathione reductase [38]. Herein, we assessed the differential effects of NSBE on GSH and GSSG levels. Our findings revealed patterns consistent with those observed for intracellular ROS. Under basal conditions, NSBE increased the cellular redox balance (GSH/GSSG ratio) above physiological levels (10-20 µg GAE/mL), mainly increasing GSH and decreasing GSSG in Caco-2 cells. Under H_2O_2 challenge, however, the redox status remained comparable to that of the positive control **(Figure 2D-F)**. Cellular redox imbalance is often interpreted as a decrease in intracellular GSH levels and/or in the GSH/GSSG ratio, as observed in CCD-18co cells [39]. In this cell line, we observed a reduction in the redox state both in the presence and absence of oxidative stress induction (**Figure 2G-I**), thereby reinforcing the pro-oxidant effect previously shown in the intracellular ROS assay.

Interestingly, the structural features of phenolic compounds confer dual-faced properties, enabling them to act as either pro-oxidant or antioxidant agents depending on factors such as concentration, pH, and the availability of oxygen and transition-metal ions. Although a pro-oxidant response is typically reported in cancer cells (attributed to their elevated intracellular copper levels) [2,40], the behaviour observed in our study was different. This ambiguous response may be attributed to the enhanced antioxidant capacity of tumour cells, driven by the up-regulation of their scavenging systems as well as by their ability to control ROS fluxes across the plasma membrane [40,41]. Thus, the dynamic maintenance of redox homeostasis in Caco-2 cells may have attenuated the pro-oxidant effects of NSBE, indicating that its cytotoxic selectivity towards Caco-2 cells is likely driven by ROS-independent mechanisms.

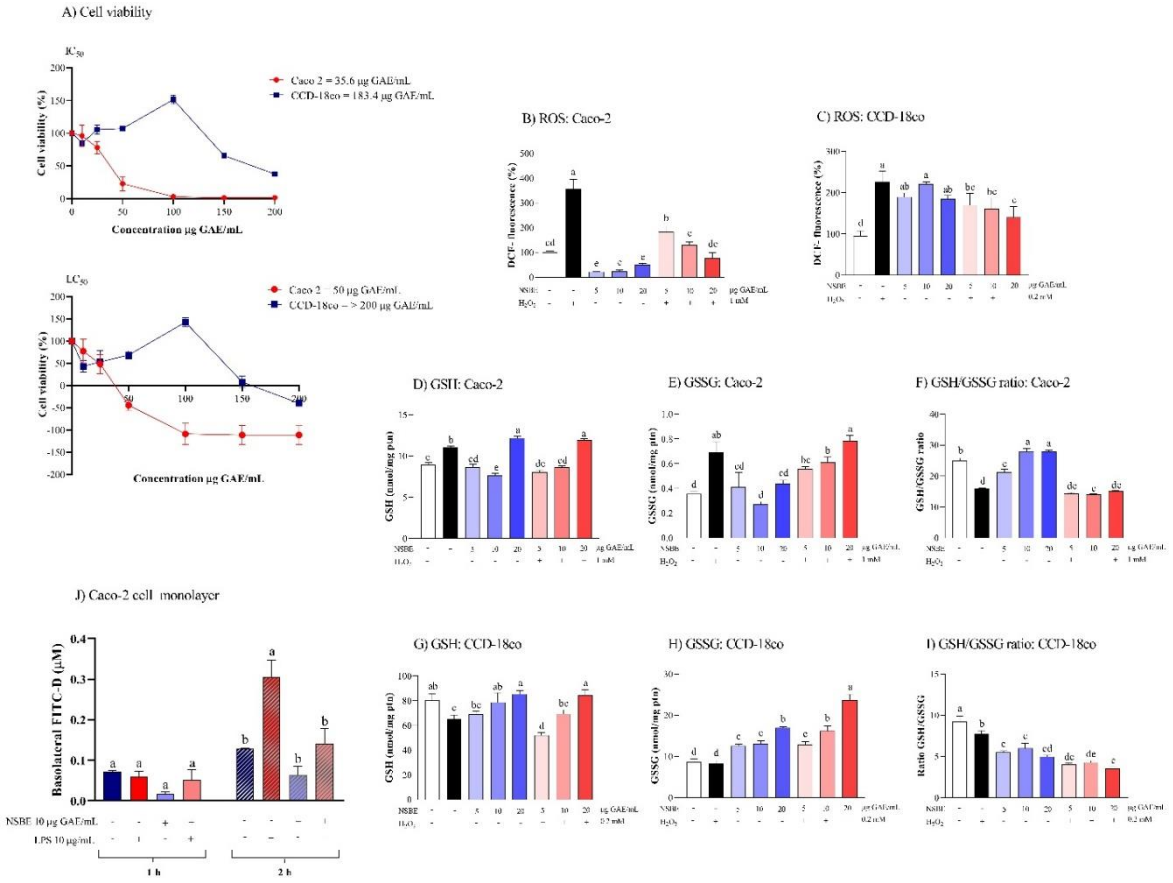


Figure 2. Effects of Norway spruce byproduct extract (NSBE) on cell viability, oxidative status, and intestinal barrier function in Caco-2. (A) Cell viability and evaluation of the concentration-dependent effect of NSBE after 48 h exposure in Caco-2 and CCD-18co cells, used to estimate IC_{50} (concentration reducing cell viability by 50%) and LC_{50} (concentration causing 50% cell death). (B, C) Intracellular ROS levels in Caco-2 and CCD-18co cells measured by DCF-based spectrofluorimetric analysis after treatment with NSBE (5–20 µg GAE/mL). (D, G) Reduced glutathione (GSH); (E, H) oxidized glutathione (GSSG); (F, I) GSH/GSSG ratio in Caco-2 and CCD-18co cells, respectively, after treatment with NSBE (5–20 µg GAE/mL). (J) Effect of NSBE on the paracellular permeability of Caco-2 monolayers. FITC-dextran permeability was assessed in Caco-2 monolayers pretreated with NSBE (10 µg GAE/mL, 24 h) and subsequently exposed to LPS (10 µg/mL, 24 h) in the continued presence of NSBE, or in the absence of LPS. One and two hours after FITC-dextran was added to the apical compartment of the transwell, 100 µL samples were collected from the basal side, and fluorescence was measured. Data are mean ± SD; values with different letters differ significantly (Tukey's test, $p < 0.05$).

3.3 Evaluation of intestinal barrier integrity by Caco-2 cell monolayer

The Caco-2 cell line is widely used as an *in vitro* model of intestinal barrier function, as it differentiates into a polarised monolayer exhibiting key enterocyte features, including an apical brush border with microvilli and tight junctions [42]. Building on this model, we investigated the protective effect of NSBE in maintaining epithelial integrity under LPS-induced inflammatory conditions, as well as its independent effect.

Consistent with barrier disruption, LPS stimulation increased paracellular permeability to FITC-dextran after 2 h of incubation with the paracellular marker (**Figure 2J**). This effect was not mirrored in the TEER values, which remained unchanged after LPS exposure (767-

1043 Ω·cm²). Paracellular permeability may occur through two distinct pathways: a) the pore pathway, which mediates the movement of small ions and solutes and is commonly assessed by TEER, and b) the leak pathway, which mediates the passage of larger solutes such as FITC-dextran. Both routes are located at the level of tight junctions; therefore, any opening that permits macromolecule passage will also allow small ions and solutes to cross [43]. We hypothesize that LPS-induced inflammation caused localised barrier disruptions among a limited number of cells within the monolayer, resulting in a detectable increase in macromolecule permeability, whose low baseline facilitates detection of changes relative to small ions and solutes [44].

Notably, NSBE preserved intestinal barrier integrity under LPS-induced challenge, as demonstrated by reduced FITC-dextran translocation to levels comparable to the negative control, while exerting no effect on epithelial permeability under basal conditions. LPS exposure is associated with increased epithelial permeability, partly due to the downregulation of tight junction proteins, along with enhanced inflammatory responses and oxidative stress [45,46]. In this context, TNF-α plays a central role in promoting leak pathway–mediated paracellular permeability through the activation of myosin light chain kinases (MLCK) [44,47]. Accordingly, our findings suggest that the protective effect of NSBE on intestinal barrier integrity likely involves both the modulation of pro-inflammatory cytokines, including TNF-α, as observed in colitis-induced colons (**Figure 5**), and the attenuation of reactive oxygen species (ROS), as evidenced in H_2O_2-stressed Caco-2 cells (**Figure 2B**).

3.4 In vivo experiment: nutritional parameters, assessment of disease activity, colonic permeability, and sickness behaviour

Analysis of nutritional parameters, DAI, behavioural assessment, and relative spleen weight demonstrated that DSS acted as a potent inducer of the inflammatory response in the animals, as discussed below (**Table 1, Figure 3**). Throughout the experiment (21 days), parameters such as body mass gain and food efficiency rate were affected by DSS treatment, which was reflected in SGR and MGR (**Table 1**). Interestingly, these differences were observed during the 5-day disease induction period, indicating that the animals' parameters were affected only during colitis induction. Treatment with NSBE managed to restore these parameters, although it did not protect the animals against the symptoms of the DAI.

Daily DAI is a key parameter in the DSS-induced colitis model, integrating weight loss, stool consistency, and faecal blood to assess disease severity, thus allowing daily monitoring of disease progression and correlating with underlying inflammatory, histological changes, and

splenomegaly, and the higher scores indicated more severe disease symptoms [48,49]. Our results revealed that DSS administration induced a significant increase in colitis severity in the DSS and DSS+ 3% NSBE groups compared to the control and NSBE groups. Gradually increased DAI scores starting from day 3 and exerted pronounced effects during the final days of treatment, suggesting that colitis severity is directly associated with the duration of DSS exposure (**Figure 3A**). Regarding disease progression, treatment with 3% DSS also led to the onset of occult bleeding (blood in the faeces) and haemorrhage, as evidenced by perianal bleeding and the presence of erythrocytes on the colonic mucosal surface observed by SEM (**Figure 3B**), in addition to marked colon shortening (**Figure 3C**).

It is also worth noting that the inflammatory process triggered by DSS is known to promote systemic immune activation, leading to behavioural alterations commonly referred to as sickness behaviour, evidenced by reduced locomotion and increased immobility time in the OFT observed in DSS-treated animals [50]. As demonstrated in **Figure 3D**, mice exposed to DSS exhibited a shorter travelled distance and longer immobility time compared to controls, indicating reduced exploratory behaviour and motor activity. Such changes are consistent with the impact of peripheral inflammation on the central nervous system, reinforcing the link between intestinal inflammation and sickness-related behavioural responses [50]. Moreover, the immune response to inflammation plays a key role in the development of UC, mostly because abnormalities of the immune organs, such as splenomegaly (**Figure 3E**), can often be detected [51], as observed in animals that received 3% DSS and NSBE + 3% DSS. In contrast, relative liver weight (**Figure 3F**) did not differ among the experimental groups, despite inflammatory and oxidative alterations observed.

The intestinal barrier is another important parameter changed by the DSS treatment, and its integrity is crucial for defending and maintaining the body's health [51]. In our study, FITC-dextran was directly injected into the intestinal lumen to assess intestinal barrier integrity and evaluate colonic permeability *in situ*. The intraluminal administration of FITC-dextran demonstrated that NSBE did not exert a protective effect on the colonic epithelium against DSS-induced injury, as evidenced by the significant increase in FITC-dextran concentrations in the plasma of colitis-induced animals (**Figure 3G**). So far, the lack of protective effect of NSBE on DAI suggests that, although the extract may have other beneficial properties, it was not sufficient to attenuate the acute clinical symptoms induced by DSS in the experimental model used, presenting a double-edged sword effect.

Table 1 - Effect of diets on mice mass gain, food intake, and metabolic parameters in 21 days of experiment.

Group	Initial mass (g/mouse)	Final mass (g/mouse)	Mass gain (g/mouse)	Food consumption (g/mouse/day)	BMG (%/mouse)	FER	SGR (% per day/mouse)	MGR ($g/kg^{0.8}$ /day/mouse)
Control	$19.7 \pm 0.9^{n.s.}$	23.2 ± 0.8^{a}	3.7 ± 0.7^{a}	$3.4 \pm 0.2^{n.s.}$	20.3 ± 2.2^{a}	0.05 ± 0.01^{a}	0.8 ± 0.2^{a}	4.1 ± 0.4^{a}
3% DSS	$19.1 \pm 1.2^{n.s.}$	21.5 ± 0.9^{b}	2.0 ± 0.7^{b}	$3.1 \pm 0.3^{n.s.}$	10.8 ± 4.1^{b}	0.03 ± 0.01^{b}	0.5 ± 0.2^{b}	2.5 ± 0.6^{b}
NSBE	$19.1 \pm 1.5^{n.s.}$	22.3 ± 1.3^{ab}	3.4 ± 0.4^{a}	$3.4 \pm 0.2^{n.s.}$	18 ± 3.4^{a}	0.05 ± 0.006^{a}	0.8 ± 0.1^{a}	3.6 ± 0.6^{a}
NSBE + 3% DSS	$18.9 \pm 1.4^{n.s.}$	22 ± 1^{ab}	3.1 ± 0.7^{a}	$3.1 \pm 0.2^{n.s.}$	17.9 ± 3.9^{a}	0.05 ± 0.01^{a}	0.7 ± 0.2^{a}	3.6 ± 0.7^{a}

Note: NSBE, Norway spruce byproduct extract (400 mg/kg/day); DSS, Dextran sodium sulphate; BMG, body mass gain; SGR, specific growth rate; MGR, metabolic growth rate; FER, food efficiency rate; All values are mean ± standard error of the mean (n = 8 per group). Values with different letters in a column are significantly different ($p < 0.05$) and n.s. represents non-significant differences between groups.

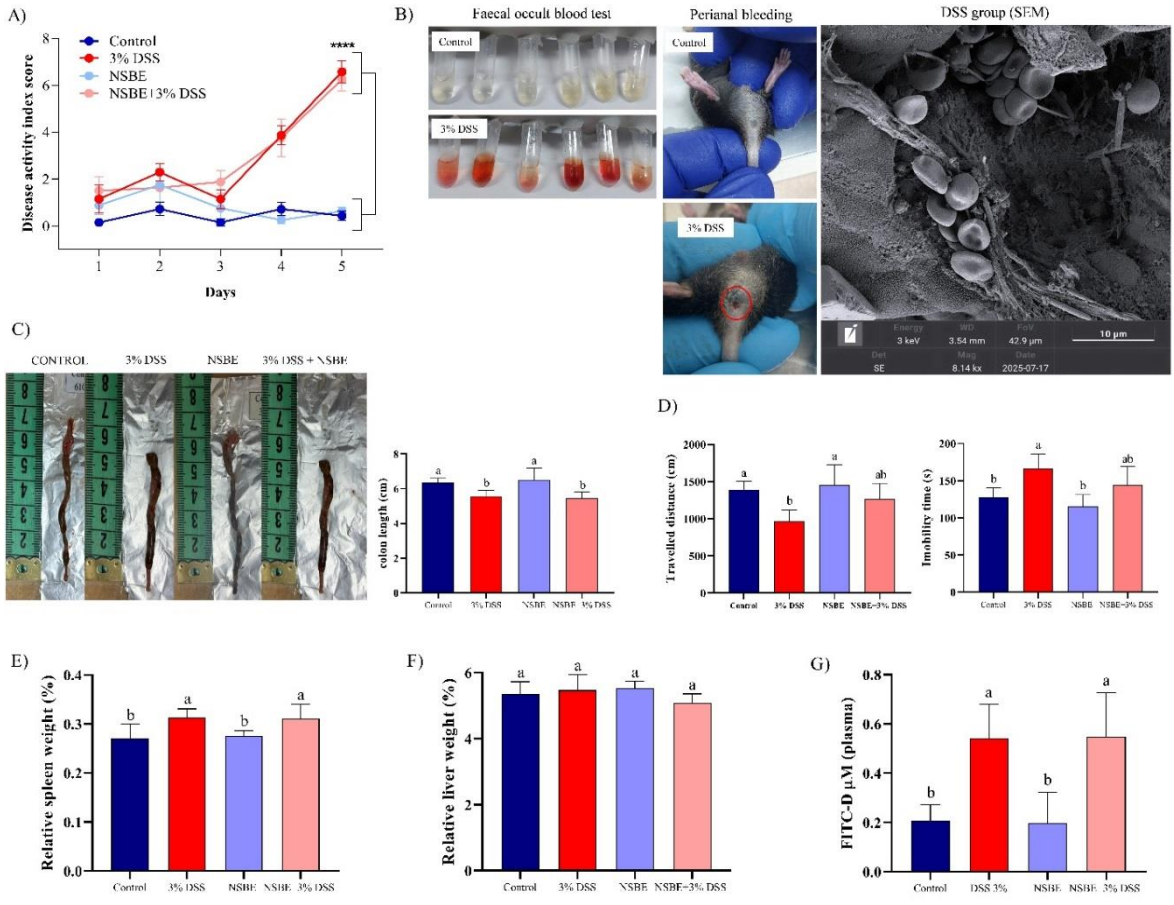


Figure 3. NSBE, Norway spruce byproduct extract (400 mg/kg/day); DSS, Dextran sodium sulphate. Effect of NSBE on DSS-induced colitis in mice. (A) Disease activity index score; (B) Presence of occult blood in faeces detected by the Kastle-Mayer test during the first days of colitis induction, and haemorrhage appearing on the final

day of induction in the 3% DSS group; with SEM of the luminal surface of the colonic mucosa showing disruption of mucosal architecture, mucus accumulation, and numerous erythrocytes on the epithelial surface. Scale bar = 10 μm. (C) Colon length; (D) Distance travelled and immobility time in the open field test; (E) Relative spleen weight; (F) Relative liver weight; (G) FITC-dextran concentrations measured 1 h after intraluminal colonic administration; Data are presented as mean ± SD (n = 8 per group). Different letters represent statistical differences (Tukey's test, $p < 0.05$).

3.5 Evaluation of DSS treatment in colon histomorphometry

Bearing in mind that DSS acts as an acute inducer of colonic and systemic inflammation and leads to visible alterations in animal nutrition and behaviour, histopathological analysis revealed well-preserved intestinal mucosa, with Lieberkühn crypts surrounded by lamina propria of moderate cellularity across all groups (**Figure 4A**).

Evident intestinal crypts with well-defined lining epithelium with a wide and homogeneous distribution of goblet cells were observed in the control and NSBE group. This histological profile indicates a preserved mucosal architecture and integrity, characteristic of a healthy intestinal mucosa [52]. Nevertheless, mucosa hypotrophy, lining epithelium thickening, and increased lamina propria cellularity were observed in 3% DSS and 3% DSS+NSBE. Moreover, marked goblet cell depletion and smaller and wider intestinal crypts with goblet cell depletion were observed in 3% DSS+NSBE and especially in 3% DSS. These cells are essential for mucin secretion into the intestinal lumen [14], explaining the depletion of mucin storage observed in 3% DSS and 3% DSS+NSBE groups (**Figure 4B**). Conversely, increased mucin distribution, known as the first physical barrier at the gastrointestinal surface [10], was detected in the control and NSBE group, indicating a predominance of Lieberkuhn crypts with high mucin production, as well as low mucin production in 3% DSS and NSBE+3% DSS groups.

In quantitative terms, the microstructural analysis reinforced the histopathological findings, indicating reduced thickness of the intestinal mucosa in 3% DSS compared to the other groups (**Figure 4C**). Crypt number, width, epithelium height, and mucin distribution were similarly reduced in 3% DSS and NSBE+3% DSS compared to the control and NSBE, which exhibited similar results. It is well known that dysregulation in colon physiology and deficiency of mucin, especially mucin 2 (MUC2), leads to inflammation and increased susceptibility to gastrointestinal tract diseases, such as inflammatory bowel disease (IBD) [14,52]. DSS is cytotoxic to goblet cells, reducing production and thinning the mucus layer, which compromises the intestinal barrier and increases epithelial exposure to the microbiota, thereby exacerbating colonic inflammation [53]. Furthermore, in the gastrointestinal tract, certain gut bacteria metabolize elements of the mucus layer, which in turn affects mucus secretion and shapes the

composition of the gut microbiota [52], as observed in our mice faecal microbiota profile (**Section 3.9**).

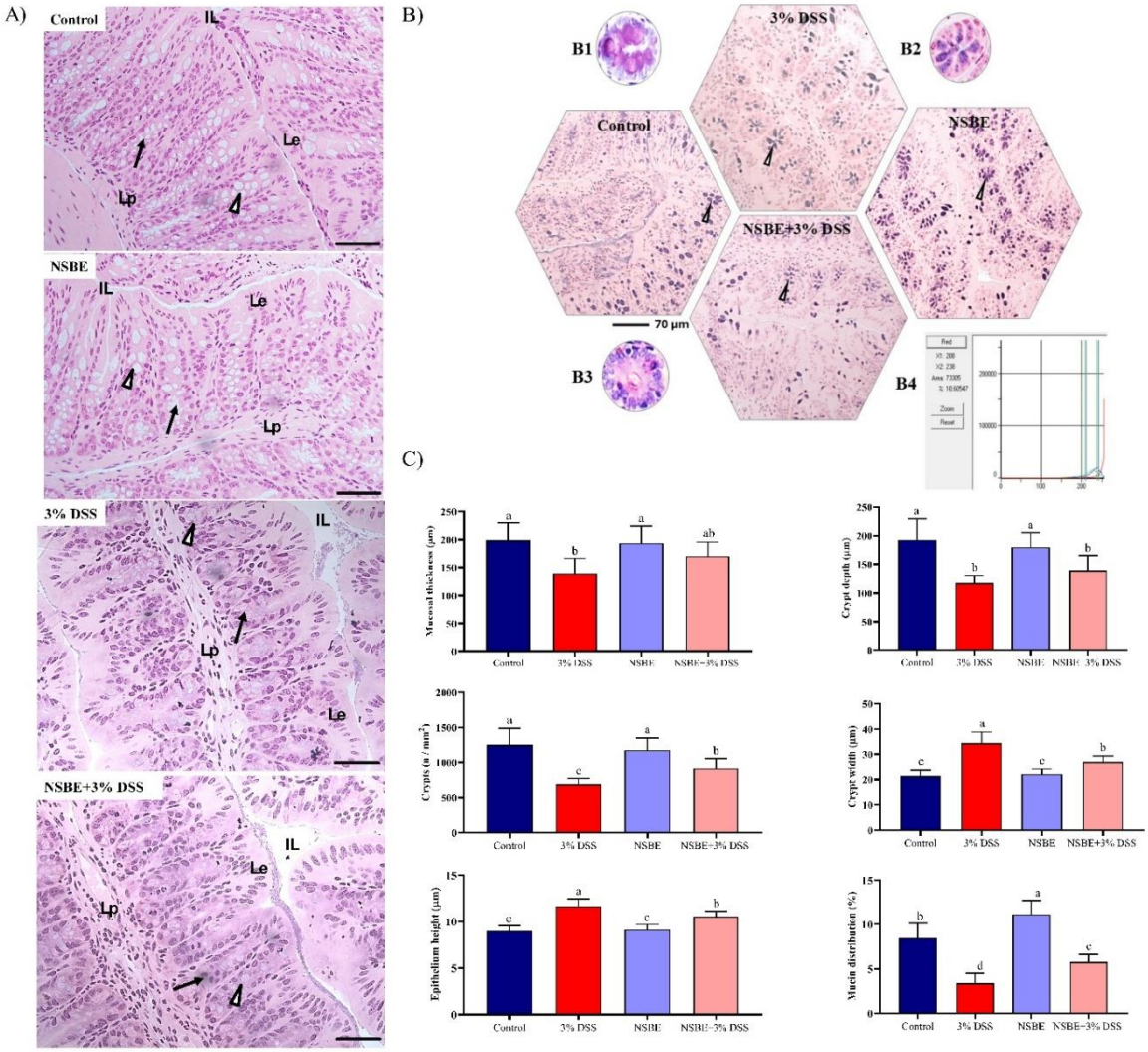


Figure 4. Histological evaluation of the colon in DSS-induced colitis following treatment with Norway spruce byproduct extract (NSBE; 400 mg/kg/day). (A) Microscopic images of the colon of animals treated with DSS and NSBE (brightfield microscopy, haematoxylin and eosin staining, scale bar = 50 µm). Arrowhead: globet cells. Lp: lamina propria (loose connective tissue). Head = intestinal crypts. IL = intestinal lumen. Le = lining epithelium. (B) Microscopic images revealing mucin distribution in the intestinal crypts of animals treated with DSS and NSBE (brightfield microscopy, haematoxylin and eosin staining, scale bar = 50 µm) (brightfield microscopy, alcian blue staining with haematoxylin counterstaining). Arrowheads: mucin storage (purple-magenta colour). Cross-sectioned intestinal crypts with high (B1), medium (B2), and low (B3) mucin production can be observed in detail. Mucin quantification was based on a histogram-based computational method, as indicated in B4. (C) Colon microstructure in animals treated with DSS and NSBE. Data are expressed as mean ± SD (n = 8 per group). Different letters in the columns indicate statistical difference among the groups (p value < 0.05).

3.6 NSBE mitigates inflammation in the colon and liver by decreasing TNF-α and IL-6 and modulating IL-10 expression in DSS-induced colitis

DSS-induced colitis triggered a marked upregulation of *TNF-α* and *IL-6* mRNA expression in the colon (**Figure 5A-B**). TNF-α plays a central role in acute colitis by activating NF-κB signalling, thereby driving the overexpression of additional pro-inflammatory cytokines genes, including *IL-6*, and establishing an inflammatory feedback loop that contributes to persistent intestinal mucosal injury. Elevated IL-6 levels, in turn, inhibit MUC2 mucin synthesis, leading to a reduction in goblet cell numbers [14], consistent with the histological findings observed in **Figure 4C**. Our experimental findings revealed a close relationship between acute intestinal inflammation and systemic effects in DSS-treated groups, reflected by splenomegaly (**Figure 3E**) and elevated hepatic *TNF-α* and *IL-6* expression (**Figure 5D-E**). This underscores the functional significance of the gut–liver axis as a bidirectional pathway linking intestinal integrity to hepatic immune responses [54].

NSBE treatment in DSS-induced colitis groups resulted in a reduction in *TNF-α* and *IL-6* expression in both colonic and hepatic tissues compared with the DSS 3% group. These effects may be attributed to phenolic constituents of the extract, particularly epicatechin [2], previously reported to suppress TNF-α and IL-6 production in a DSS-induced colitis model [55]. Indeed, interventions capable of limiting pro-inflammatory cytokine production, particularly TNF-α, are considered highly relevant in the management of intestinal inflammation, given the pivotal role of this cytokine in mucosal damage and inflammatory amplification [13,14].

Interestingly, NSBE administration under basal conditions resulted in an upregulation of pro-inflammatory cytokines *TNF-α* and *IL-6* expression compared with controls, which was not accompanied by inflammatory histomorphology alterations in colonic tissue. This response may be associated with a shift towards a more oxidised intracellular redox state observed in this group (**Section 3.7**). Such redox imbalance may modulate redox-sensitive signalling pathways, including NF-κB and MAPK, thereby interfering with the expression of pro-inflammatory cytokines [56]. Within this cytokine-driven context, the divergent responses observed under basal and DSS-induced conditions indicate a context-dependent inflammatory action of NSBE.

Beyond the effects of pro-inflammatory cytokines, the resolution of intestinal inflammation also relies on anti-inflammatory mediators, among which IL-10 plays a key role in maintaining gastrointestinal integrity by limiting tissue damage [31]. As shown in **Figure 5C/F**, *IL-10* expression was upregulated in both liver and colon tissues of the 3% DSS group, contrasted with the typical inflammatory profile described in acute DSS-induced colitis, where *IL-10* expression is often reduced [9,53]. This discrepancy suggests a compensatory upregulation of IL-10 in response to the inflammatory state triggered by DSS, although often,

this response is not enough to completely contain the damage [57]. Taken together, these observations show that *IL-10* levels reflect the inflammatory burden induced by DSS. By reducing *TNF-α* and *IL-6* expression, NSBE supplementation in colitis likely attenuated IL-10-mediated counter-regulation, resulting in *IL-10* downregulation in both the colon and liver.

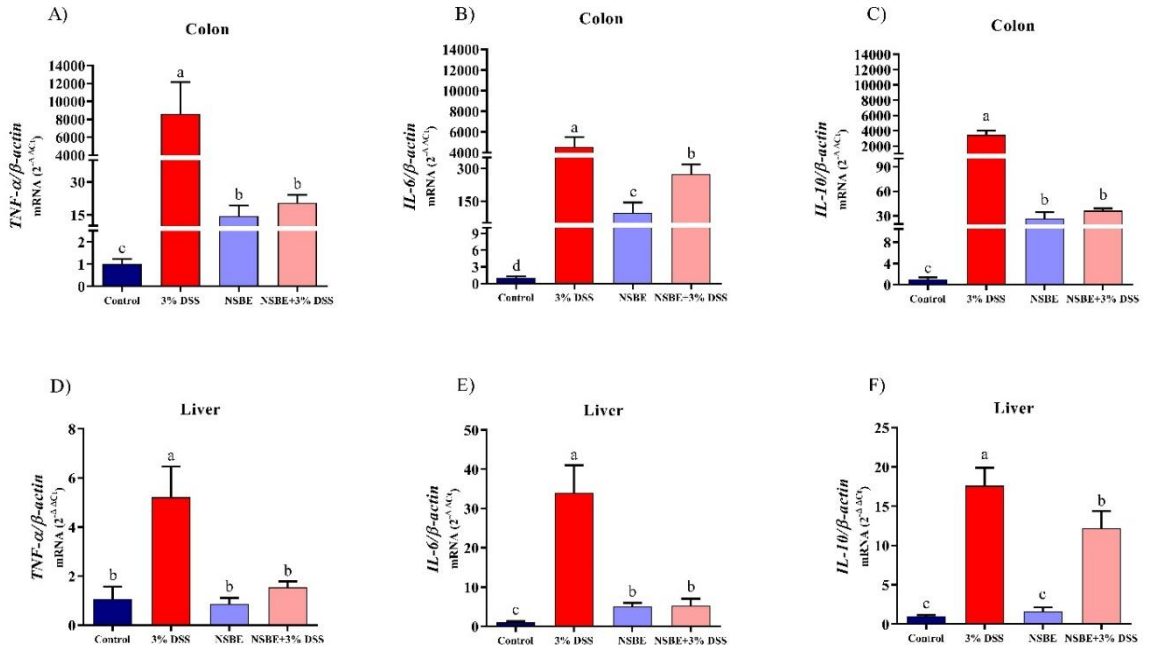


Figure 5. NSBE, Norway spruce byproduct extract (400 mg/kg/day). NSBE reduces the expression of pro-inflammatory cytokines *TNF-α* (A, D) and *IL-6* (B, E) in the colon and liver, respectively, while modulating *IL-10* expression (C, F). Error bars represent the mean ± SD of triplicate analysed samples (n = 8 per group). Different letters represent statistical differences (Tukey's test, $p < 0.05$).

3.7 Differential redox responses to DSS and NSBE in colon and liver tissues

Oxidative stress plays a central role in the initiation and progression of intestinal inflammatory disorders. This redox imbalance promotes extensive cellular damage, largely through peroxidation of polyunsaturated fatty acids (PUFAs) and the formation of lipid peroxides and their breakdown products, including malondialdehyde (MDA) [58]. To elucidate the anti-oxidative effects of NSBE in the context of ulcerative colitis, MDA levels were quantified using the thiobarbituric acid-reactive substances assay. As illustrated in **Figure 6A**, NSBE treatment in the colitis-induced group restored colonic MDA concentrations to baseline levels. However, this protective effect was not observed in the liver, where MDA levels remained comparable to those of the DSS 3% group **Figure 6B**. This differential response likely reflects the predominantly local action of the polyphenolic compounds present in NSBE within

the inflamed intestinal lumen, as their reduced bioaccessibility after digestion prolongs their residence in the colon and thereby enhances their local effects [2,59]

During lipid peroxidation, hydroperoxy groups are introduced into PUFA chains, disrupting lipid–lipid and lipid–protein interactions, which compromise membrane integrity and exacerbate inflammation [58]. To mitigate oxidative damage and preserve redox balance, cells rely on antioxidant defences, including non-enzymatic molecules (GSH) and enzymatic systems (SOD, GPx, GR, and catalase) [31]. Herein, we evaluated glutathione levels, given its role in reducing organic hydroperoxides and lipid peroxides. In the colon, the NSBE + 3% DSS group prevented MDA accumulation and reduced pro-inflammatory cytokine levels, yet GSH and GSSG levels **(Figure 6C-D)** remained similar to the DSS 3% group, indicating that the extract mitigates oxidative damage without restoring the glutathione redox balance **(Figure 6E)**. Moreover, in the liver, although GSH levels increased in the NSBE + 3% DSS group **(Figure 6F)**, this group exhibited a 91% reduction in the GSH/GSSG ratio relative to the control **(Figure 6H)**, reflecting pronounced intracellular oxidative stress **(Figure 6G)**.

NSBE administration under homeostatic conditions resulted in a reduction in the GSH/GSSG ratio in both colonic and hepatic tissues without inducing lipid peroxidation or causing histopathological alterations in the colon, relative to the control group. This dual response, also evident in the expression of pro-inflammatory cytokines **(Section 3.6)**, is consistent with adaptive redox modulation, whereby phenolic constituents elicit mild oxidative perturbations that stimulate endogenous defence mechanisms.

The pro-oxidant activity of phenolic compounds has been associated with the oxidation of catechol groups, such as those present in epicatechin, leading to the formation of electrophilic o-quinones that can undergo redox cycling and promote reactive oxygen species generation [60,61]. These quinones can react with glutathione (GSH) and also be oxidized to GSSG by hydroxyl radicals generated during the redox cycle, thereby supporting the redox imbalance observed in the liver and colon [61]. Thus, upon sensing electrophilic stress, Keap1 releases NRF2, which translocates to the nucleus and binds to the ARE, strongly inducing the transcription of phase II antioxidant enzymes that protect cells against oxidative stress and inflammation [34]. Importantly, whether phenolic compounds act as antioxidants or pro-oxidants is highly context-dependent and influenced by factors such as pH, compound concentration, oxygen availability, and the presence of transition metals, including copper ions [40]. These parameters are dynamically modulated throughout the digestive process, potentially shaping the redox behaviour and biological effects of phenolic compounds [2].

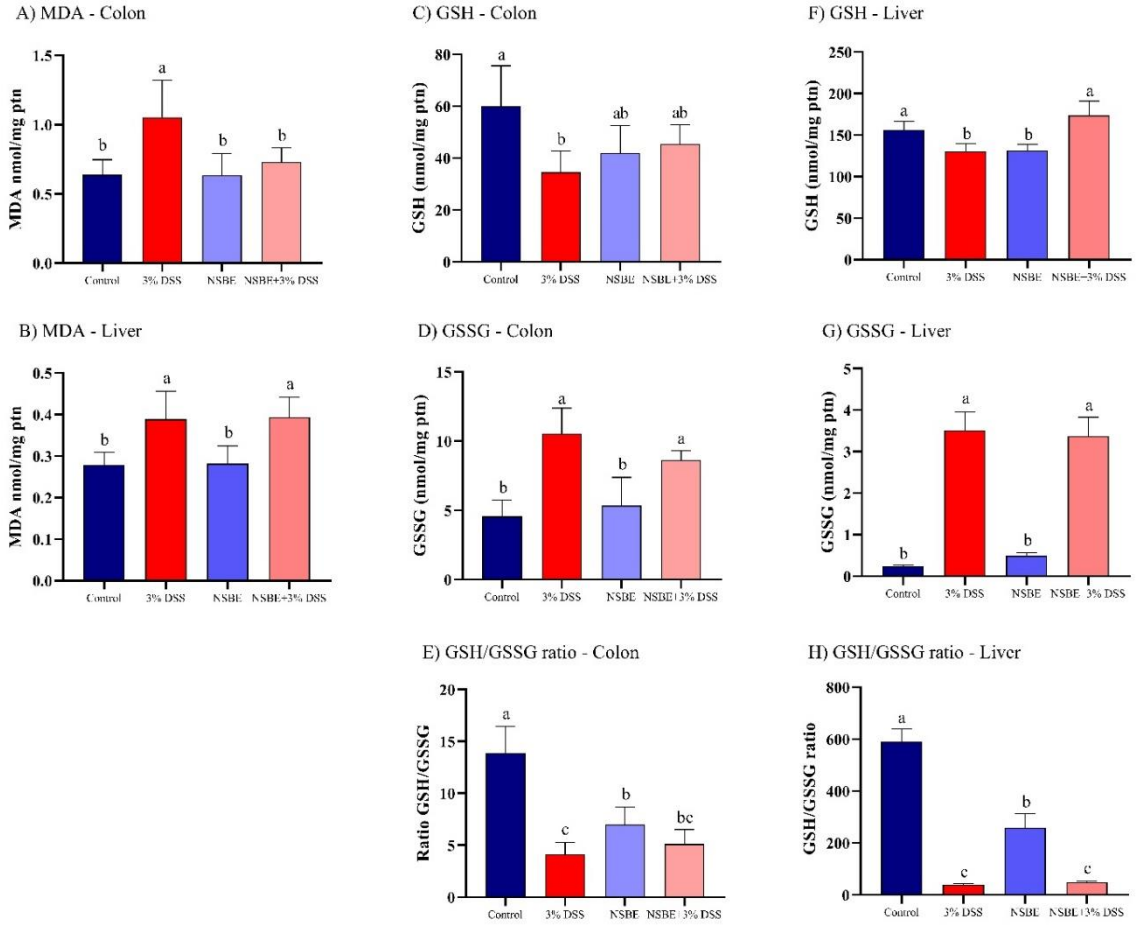


Figure 6. Effect of NSBE treatment on oxidative stress markers in the colon and liver. (A, E) GSH, reduced glutathione; (B, F) GSSG, oxidized glutathione; (C, G) GSH/GSSG ratio; (D, H) MDA, malondialdehyde. All values are expressed as mean ± SD (n = 8 per group). Different letters indicate statistically significant differences (Tukey's test, $p < 0.05$).

3.8 NSBE attenuates inflammatory responses by reducing IFN-γ and IL-17A while promoting IL-10 production in splenocytes

To evaluate the immunomodulatory effects of NSBE, the levels of IFN-γ, IL-17A, and IL-10 were quantified in splenocyte cultures after 24 h under spontaneous conditions (medium alone) or following stimulation with NSBE, LPS, or NSBE+LPS (**Figure 7A-C**). These cytokines were used as representative markers of T-helper (Th) cell–mediated immune responses, corresponding to Th1, Th17, and regulatory responses, respectively [62].

The increased spontaneous production of IFN-γ and IL-17A, together with reduced IL-10 levels observed in splenocytes from DSS-treated animals, indicates a systemic pro-inflammatory immune profile associated with intestinal injury (**Figure 3E**). Indeed, intestinal inflammation–induced epithelial barrier disruption leads to systemic immune responses in the spleen [63], leading to activation of Th1 and Th17 pathways, which contribute to the amplification of inflammatory responses [43,62].

Splenocytes from the NSBE+DSS group also exhibited elevated IFN-γ and IL-17A levels under basal conditions, but this response was accompanied by increased IL-10 secretion. IL-10 is a key regulatory cytokine that plays a central role in limiting excessive inflammation by suppressing the production of pro-inflammatory mediators and promoting immune homeostasis [14]. The enhanced IL-10 production observed in NSBE-treated animals therefore suggests that the extract may favour the establishment of a more regulated systemic immune environment during colitis. This hypothesis is further supported by the observation that IL-10 production remained elevated in the NSBE+DSS group following stimulation with NSBE or LPS, indicating that immune cells from treated animals retain an increased capacity to produce anti-inflammatory mediators under inflammatory stimuli.

Moreover, NSBE modulated systemic immune responses in colitis by reducing Th1- and Th17-associated responses, as evidenced by reduced production of IFN-γ and IL-17A in the NSBE+DSS group compared with the DSS group following *ex vivo* stimulation with NSBE, LPS, or both. The immunomodulatory effects of NSBE observed in splenocytes may reflect modulation of the gut microbiota and reduced exposure to pro-inflammatory signals [64]. Such mechanisms could contribute to restoring immune balance and limiting the propagation of intestinal inflammation to peripheral immune organs.

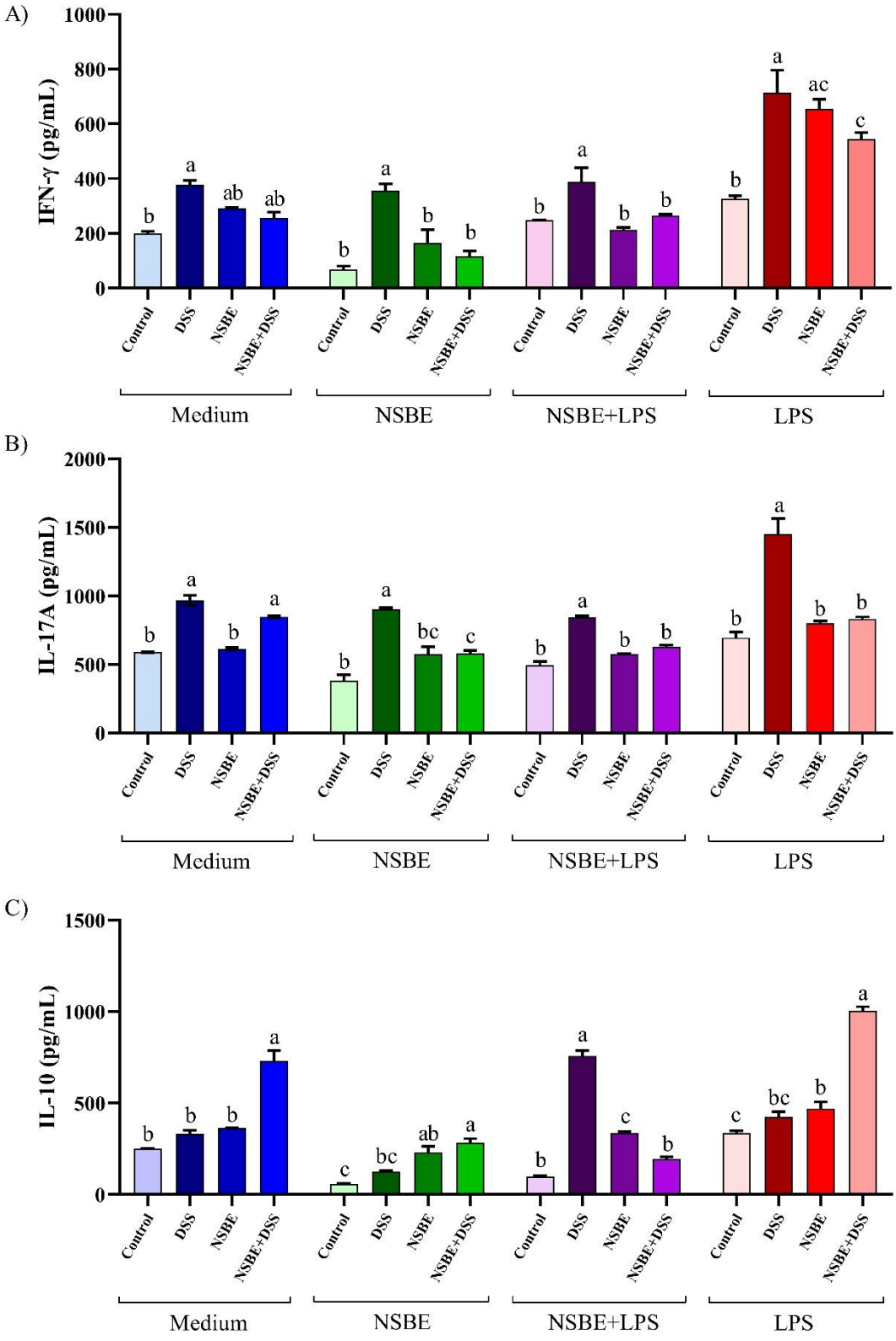


Figure 7. Cytokine production of IFN-γ (A), IL-17A (B), and IL-10 (C) in splenocyte cultures after 24 h of ex vivo stimulation. Cells were cultured with medium alone or stimulated with NSBE, NSBE+LPS or LPS. Cytokine levels were quantified by ELISA and expressed in pg/mL. Error bars represent the mean ± SD of triplicate samples (n = 8 per group). Different letters indicate statistically significant differences within the same stimulation condition (medium, NSBE, NSBE+LPS, or LPS), according to Tukey's test ($p < 0.05$).

3.9 Effect of NSBE on faecal gut microbiota, SCFA production, and bacterial translocation to mesenteric lymph nodes

The intestinal microbiota, under normal physiological conditions, orchestrates multiple gut metabolisms, such as pathogen exclusion, maintenance of mucosal integrity, and modulation of host immunity [11]. This microbiota is responsible for the SCFAs production formed by fermentation of undigested carbohydrates in the caecum and colon, acting as central mediators of host–microbiota interactions [15,65]. In this context, we assessed the effect of NSBE on microbiota modulation and its impact on SCFAs (acetic, propionic, isobutyric, butyric, isovaleric, and valeric acids) in the presence and absence of 3% DSS-induced colitis.

As observed in our results, DSS treatment reduced total SCFA production (**Figure 8A**), accompanied by a decreased abundance of *Bacteroidetes* (Gram-negative) and *Firmicutes* (Gram-positive) (**Figure 8C**), the dominant fibre-fermenting phyla in the healthy gut [66,67]. Alterations in the *Firmicutes*/*Bacteroidetes* (F/B) ratio are closely linked to gut dysbiosis and inflammatory bowel disease, which turn increase epithelial oxygenation and favour the dysbiotic expansion of facultative anaerobic *Proteobacteria* [31,68]. Based on our findings, 3% DSS administration induced a dysbiotic state characterised by an approximately 80% reduction in the F/B ratio relative to the control group and a pronounced expansion of *Gammaproteobacteria*, a potentially pathogenic class of Proteobacteria, with limited fibre-fermenting capacity [31]. In addition, the 3% DSS group showed an increase in total bacterial abundance, driven by the overgrowth of *Gammaproteobacteria*, suggesting that DSS-induced dysbiosis results from the loss and imbalance of SCFA-producing taxa. This profile directly contributes to reduced SCFA production together with the opportunistic expansion of pathogenic *Proteobacteria*, creating a microbial landscape conducive to intestinal inflammation, as previously observed by Zhuang et al. [69].

The ability of NSBE to restore SCFA production under colitic conditions reflects its capacity to modulate gut microbial composition, as evidenced by increased abundance of *Bacteroidetes* and reduced *Gammaproteobacteria* (**Figure 8C**). This effect results from the combined action of its constituents, where mannose and soluble fibres, such as galactoglucomannan, serve as substrates for SCFA-producing taxa, while phenolic compounds (epicatechin), exert prebiotic effects and concurrently suppress the expansion of inflammation [5,70,71]. Despite these changes, NSBE did not fully restore the *Firmicutes/Bacteroidetes* balance, which remained around 50% lower than in healthy animals, suggesting a residual dysbiotic and pro-inflammatory state.

Among the SCFAs analysed, acetic acid was identified as the most abundant fatty acid (**Figure 8B**) and possibly the main contributor to the protective effect of GGM during colitis. This effect was possible through the restoration of the abundance of *Bacteroidetes* to control group levels, once this phylum is the main producer of acetic acid [66], contributing to the observed protective effect. In contrast, DSS treatment specifically reduced the levels of acetic and butyric acids, while propionic and valeric acid levels were not affected. This variability under colitic conditions is commonly observed in studies, as well as the abundance of specific microbial taxa [65]. Concerning the branched-chain short-chain fatty acids (BCFA), we observed distinct patterns: isobutyric acid levels in the 3% DSS group were higher than those in the control group, whereas isovaleric acid showed no difference between them. This behaviour differs from most studies, in which isobutyric acid is not typically increased under colitic conditions [72], highlighting the uniqueness of our findings. Although NSBE administration in healthy mice did not alter overall SCFA profiles, it reduced acetic acid levels relative to the control group, without affecting total SCFA content, likely compensated by the production of other SCFAs. Notably, isovaleric and valeric acid levels increased under these conditions. Together, these results suggest that, even under healthy conditions, NSBE may modulate specific SCFAs.

Building on these insights, we assessed bacterial translocation to mesenteric lymph nodes (MLNs) under both physiological and inflammatory conditions (**Figure 8C**). We observed basal bacterial translocation into MLNs across all phyla in the Control group. Indeed, bacterial translocation can occur under homeostatic conditions in healthy mice and likely contributes to the maintenance of host immunity by delivering small amounts of bacteria and bacterial components to the mononuclear phagocyte system (MPS) in the liver and MLNs, organs that act as ‘firewalls’ against invading microorganisms [64,73]. Notably, the *Bacteroidetes* phylum exhibited increased translocation in the NSBE-supplemented group without induced colitis. Assessment of paracellular intestinal integrity revealed that FITC-dextran permeability in this group remained comparable to the control, indicating that this increase occurred without damage to the epithelial barrier. This likely reflects the close correlation between the composition of the faecal microbiome and that of adjacent mucosal sites [74].

In contrast, DSS-induced colitis resulted in pronounced translocation of *Gammaproteobacteria* and total bacteria, consistent with epithelial barrier disruption, as evidenced by increased FITC-dextran permeability, inflammation, mucin depletion, and dysbiosis observed in this group. Inflammation-driven alterations of the apical junctional

complex (AJC), epithelial cell apoptosis and lesions, along with mucus layer reduction, provide permissive routes for bacteria to traverse the intestinal epithelium [43,75]. Interestingly, although disruption of epithelial barrier integrity was observed, the 3% DSS+NSBE group exhibited increased translocation of *Gammaproteobacteria* despite their low faecal abundance, suggesting a high intrinsic translocation capacity of this bacterial class. Members of the *Enterobacteriaceae* family (*Escherichia coli*, *Klebsiella* spp.), which belong to the class *Gammaproteobacteria*, are among the most prevalent organisms reported in bacterial translocation in humans, likely due to their enhanced ability to adhere to the intestinal mucosa [73].

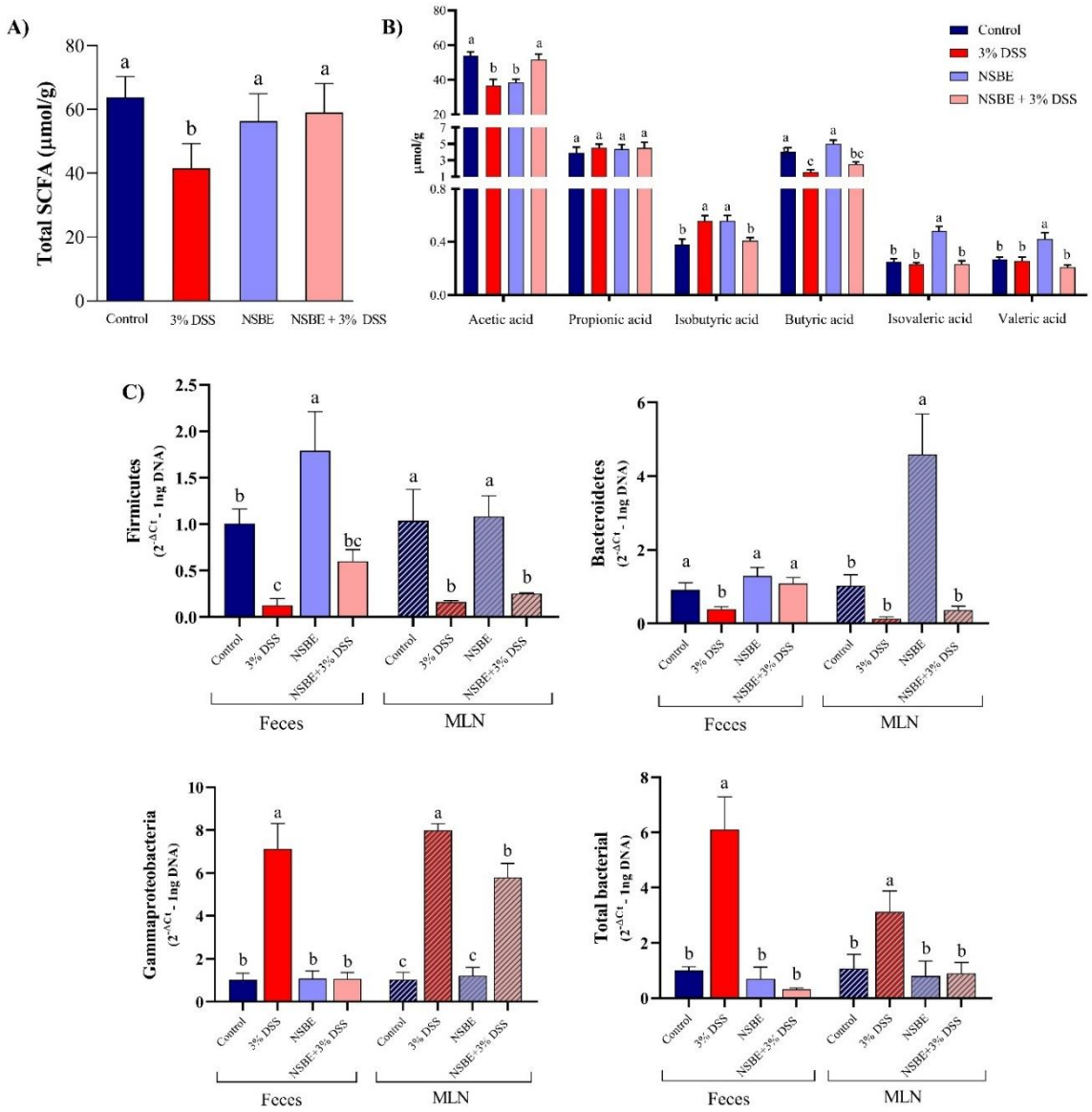


Figure 8. Modulation of gut microbiota, bacterial translocation, and short-chain fatty acid (SCFA) production by Norway spruce byproduct extract (NSBE; 400 mg/kg/day) in DSS-induced colitis. (A) Total SCFA content (µmol/g). (B) Faecal SCFA profiles, including acetic, propionic, isobutyric, butyric, isovaleric, and valeric acids

(μmol/g). (C) Relative abundance of bacterial groups in faeces and bacterial translocation to mesenteric lymph nodes (MLN), including *Firmicutes*, *Bacteroidetes*, *Gammaproteobacteria*, and total bacteria. Results are expressed as relative DNA concentrations, calculated using the $2^{-\Delta CT}$ method and normalised to the Control group. Error bars represent the mean ± SD (n = 8 per group). Different letters indicate statistically significant differences (Tukey's test, $p < 0.05$). DSS, Dextran sodium sulphate.

4. Conclusions

NSBE, a Norway spruce by-product extract, exerts dual and context-dependent effects *in vitro* and *in vivo*. In Caco-2 cells, NSBE exhibited strong cytotoxic and antiproliferative effects, reduced oxidative damage, and protected intestinal barrier integrity against LPS-induced dysfunction. On the other hand, in CCD-18Co cells, the extract exhibited a pro-oxidant profile despite its selective cytotoxicity toward Caco-2 cells. In DSS-induced colitis, NSBE supplementation failed to prevent clinical disease activity or bacterial translocation, and under homeostatic conditions, even promoted basal *Bacteroidetes* translocation, increased pro-inflammatory cytokine expression, and disrupted glutathione redox balance. Despite this, NSBE attenuated pro-inflammatory cytokine expression, normalised colonic MDA levels, and promoted the restoration of total SCFAs, associated with an increased abundance of the phylum *Bacteroidetes* and a reduction in *Gammaproteobacteria*. These results underscore the complexity of GGM-rich plant-derived by-products as nutraceutical interventions, highlighting both their immunomodulatory potential and their capacity to exacerbate dysbiosis-driven outcomes. From a translational perspective, our findings emphasise the need for rigorous safety and efficacy assessments before incorporating forestry by-products into therapeutic strategies for IBD. By bridging sustainability, circular economy principles, and biomedical relevance, this work provides critical insights into the "double-edged sword" nature of natural by-products, supporting their cautious but promising application in gut health interventions.

Author contributions

Thaise Caputo Silva: formal analysis, methodology, writing - original draft; **Amanda dos Santos Lima:** formal analysis, methodology, writing - original draft; **Fernando Vitor Vieira:** formal analysis, methodology, writing - original draft; **Nathália Alves Bento:** formal analysis, writing - original draft; **Evandro Neves Silva:** formal analysis, methodology; **Luiz Eduardo Lobo e Silva:** formal analysis, methodology; **Graziela Domingues de Almeida Lima:** formal analysis; **Petri Kilpelainen**: methodology, conceptualization; **Rômulo Dias Novaes:** formal analysis, methodology, writing - original draft, conceptualization; **Roger Wagner:** formal analysis, methodology, conceptualization; **Leonardo Augusto de Almeida:** formal analysis,

methodology, conceptualization; **Luciana Azevedo:** writing - original draft, supervision, methodology, funding acquisition, data curation, conceptualization.

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Declaration of competing interest

The authors declare that they have no competing interests.

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Supplementary material

Table S1. Primer sequences employed to assess cytokine gene expression and bacterial phyla through 16S rRNA gene targeting.

Gene	Sequences
Bacteroidetes	F: 5'-GTTTAATTCGATGATACGCGAG-3' R: 5'-TTAASCCGACACCTCACGG-3'
Firmicutes	F: 5'-GGAGYATGTGGTTTAATTCGAAGCA-3' R: 5'-AGCTGACGACAACCATGCAC-3'
Gammaproteobacteria	F: 5'-GCTAACGCATTAAGTRYCCCG-3' R: 5'-GCCATGCRGCACCTGTCT-3'
Total bacteria	F: 5'-AGAGTTTGATCCTGGCTCAG-3' R: 5'-AAGGAGGTGWTCCARCC-3'
β-actin	F: 5'-AGGTGTGCACCTTTTATTGGTCTCAA-3' R: 5'-TGTATGAAGGTTTGGTCTCCCT-3'
TNF-α	F: 5'-CATCTTCTCAAAATTCGAGTGACAA-3' R: 5'-TGGGAGTAGACAAGGTACAACCC-3'
IL-6	F: 5'-CCAGGTAGCTATGGTACTCCAGAA-3' R: 5'-GATGGATGCTACCAAACTGGA-3'
IL-10	F: 5'-GGTTGCCAAGCCTTATCGGA-3' R: 5'-ACCTGCTCCACTGCCTTGCT-3'

Note: F: forward, R: reverse. Nucleotide symbols: R = A or G; Y = C or T; S = C or G.

Table S2. Calibration curves and analytical validation parameters for short-chain fatty acids determined by GC–FID.

R^2	**Acetic acid**	**Propionic acid**	**Butyric acid**	**Isovaleric acid**
	0.9999	0.9996	0.9999	0.9999
Equation	y = 0.0336x + 0.0043	y = 0.0583x + 0.0026	y = 0.0852x + 0.0007	y = 0.1113x + 0.0003
Linear range (µmol g^{-1})*	1.01 - 24.16	0.11 - 3.46	0.04 - 5.38	0.02 - 0.50
LOD (µmol g^{-1})	0.03	0.03	0.02	0.01
LOQ (µmol g^{-1})	0.06	0.05	0.04	0.02
Accuracy (%)	96.00	102.34	90.70	92.39
Repeatability (RSD)	3.69	5.27	3.73	1.63

Note: * The linear range, LOD (limit of detection) and LOQ (limit of quantitation) were expressed in mmol of SFA per kg of faeces

4. CONSIDERAÇÕES FINAIS

O abeto-norueguês (*Picea abies*) é uma das espécies florestais de maior relevância ecológica e econômica da Europa, sendo amplamente explorado pela indústria madeireira e, consequentemente, responsável pela geração de grandes volumes de subprodutos, como a serragem. Tradicionalmente destinados a aplicações de baixo valor agregado, esses resíduos representam uma fonte ainda pouco explorada de compostos bioativos com potencial para aplicações funcionais e nutracêuticas.

A caracterização da composição centesimal do extrato liofilizado demonstrou tratar-se de uma matriz predominantemente composta por carboidratos, ricos em glicose e manose, compatíveis com a estrutura de galactoglucomananos, e contendo aproximadamente 7,5% de fibras solúveis de alto peso molecular. Esse perfil, associado à presença de compostos fenólicos, como catequina e epicatequina, corrobora dados prévios da literatura e sustenta o potencial funcional do ESAN como fonte de biomassa renovável de baixo custo com propriedades bioativas relevantes.

Nos ensaios *in vitro*, o ESAN apresentou citotoxicidade seletiva para as células tumorais Caco-2, evidenciada por valores significativamente menores de IC_{50} e LC_{50} em comparação às células não cancerosas CCD-18Co, resultando em um índice de seletividade superior a 3. O extrato também modulou de maneira distinta o balanço redox entre as duas linhagens. Nas células Caco-2, reduziu os níveis basais das espécies reativas de oxigênio e favoreceu o estado redox, enquanto em células CCD-18Co induziu um perfil pró-oxidante moderado, associado à redução da relação GSH/GSSG. Esses resultados sugerem que a seletividade citotóxica do NSBE não está diretamente relacionada à indução de estresse oxidativo, mas possivelmente à modulação de vias metabólicas e de sobrevivência específicas do fenótipo tumoral.

Embora *in vitro* o ESAN tenha demonstrado potencial para preservar a integridade da monocamada de células Caco-2, no modelo *in vivo* de colite aguda induzida por DSS o extrato não foi capaz de manter a integridade do epitélio colônico, conforme avaliado pelo marcador paracelular FITC-dextrana. Ainda que o ESAN tenha promovido a atenuação da expressão de citocinas pró-inflamatórias, como TNF-α e IL-6, e contribuído para a modulação da microbiota intestinal, com aumento da abundância de Bacteroidetes e da produção de ácido acético, não foi observada redução do índice de atividade da doença, indicando efeitos sistêmicos benéficos limitados. Por outro lado, a administração isolada do ESAN em condições de homeostase promoveu aumento da translocação bacteriana, acompanhado por elevação parcial de

marcadores inflamatórios e de estresse oxidativo, sem comprometimento da permeabilidade epitelial.

Em conjunto, esses achados evidenciam o caráter ambivalente do perfil bioativo do ESAN e reforçam a necessidade de estudos adicionais para elucidar seus mecanismos de ação, otimizar doses e estratégias de administração, bem como assegurar sua segurança antes de uma eventual aplicação no manejo de doenças inflamatórias intestinais.

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